Effects of a Fat-Sugar Supplemented Diet, With and Without Exercise Training, on Endothelial Function, Blood Pressure, and Markers of Cardiovascular Risk

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2013 by the
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May 2013

ABSTRACT

The Western Pattern diet has been characterized by having greater than 50 percent consumption coming from fat and sugar. This macronutrient allocation has been shown to have deleterious effects on endothelial function and metabolic markers of cardiovascular disease. Exercise has been shown to improve vascular reactivity and metabolic markers related to cardiovascular health. The objective of the study was to determine if exercise training can prevent the anticipated deleterious effects of a fat-sugar supplemented diet on endothelial function and blood markers of cardiovascular risk in young men. Twenty-one, healthy college-aged males were randomly assigned to either the doughnut + exercise or doughnut only groups. Both groups were fed 2 doughnuts per day, 6 days per week, for three weeks, while maintain their current diet. The exercise group completed 4 exercise training sessions per week consisting of 2 high intensity interval training bouts (up to 95% VO_2peak) on a cycle ergometer and two moderate intensity, steady-state bouts (at 75% VO_2peak) on a treadmill. Changes in body weight and composition, markers of endothelial function, oxidative stress, serum lipids, and blood glucose were measured in each group. As expected, cardiovascular fitness increased significantly in the doughnut-supplemented + exercise group as compared to the doughnut-supplemented ($p=0.005$). Significant increases in body weight ($p=0.036$), fat mass ($p=0.013$), and body fat percentage ($p=0.014$) were seen in the doughnut only group as compared to the doughnut + exercise group. The doughnut + exercise group showed significant improvements in fasting serum triglycerides ($p=0.036$), plasma insulin ($p=0.039$) and insulin sensitivity (HOMA; $p=0.05$) as compared to the doughnut only group. The doughnut + exercise group saw a significant improvement in nitric oxide availability

whereas the doughnut only group experienced a significant decline ($p=0.014$). There were no significant changes in other markers. Despite the addition of a fat/sugar supplement of ~11,600 kcal over three weeks, 4 exercise sessions per week were sufficient to prevent a gain in body weight and fat mass, and also improve some measures of cardiometabolic risk. These results suggest that exercise may be necessary to prevent some adverse health outcomes associated with transient periods of excessive energy consumption.

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my mentor, Dr. Glenn Gaesser, for the constant encouragement and valued counsel he provided throughout my doctorate experience. There were plenty of challenges that presented themselves during my time in the PANW program but no matter how many times I lost focus I could rest assured that getting back on track was just an email away – your patience with me was unswerving. Thank you for having faith in my ability to finish and being there when I found that faith in myself.

To the rest of my committee, thank you. Each of you played an integral part in successfully completing my dissertation. You were selected professionally out of respect for the mastery you have in your field and personally because of your willingness to help guide me through the dissertation process. I have gained so much from the input that each of you offered and I am so grateful for each of your contributions. The time that you dedicated as a member of this committee will never be forgotten.

You have all been invaluable during my time at ASU. I appreciate all of you very much and would be more than happy to ever return the favor of support and service that you have so graciously shown me. To my lab colleagues, I could not have done this without you. The time and effort that you put in to helping make the research happen was essential to my finishing. I could not have asked for a more cohesive and welcoming group to come into when I returned to complete my research. Individually, your insight and expertise really challenged me to be a better student, researcher and colleague. It is my hope that you too find such gratification in the time you spend pursuing your degree while at ASU.

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Chapter 1

INTRODUCTION

The Western Pattern diet, popular in many developed countries, is characterized by high intakes of red meat, sugary desserts, high-fat foods, and refined grains. Commonly included in this diet is also a large consumption of high-fat dairy and sugar-sweetened beverages. More than 50 percent of all calories consumed in America come from fat and sugar. There currently exists a plethora of research studies that confirm the deleterious effects of both high-sugar and high-fat diets on endothelial function and metabolic markers of cardiovascular disease (Nicholls et al., 2006; Johnson et al, 2009).

Over the last ten years new evidence has emerged regarding the relationship between intake of sugars and cardiovascular disease (Ceriello et al., 2002). The shift in dietary priority from nutritional value to convenience specifically over the past 40 years has been marked by a 19% rise in added sugars and an average intake of 22.2 grams of sugar per day (Johnson et al, 2009). This average consumption equates to 355 kilocalories per day which is well above the US Dietary Guidelines for discretionary calories consumed per day of 100 kilocalories for women and 150 kilocalories for men (US Department of Health and Human Services, 2005). It is well supported that individuals with diabetes have an increased risk for cardiovascular disease; however, there are some studies that suggest that postprandial hyperglycemia and hypertriglyceridemia in nondiabetics are also independent risk factors for developing cardiovascular disease (Ebenbichler et al., 1995).

In addition to a rise in sugar consumption, convenience-based dietary selections have also brought about an increase in the amount of saturated and trans fat consumed

(although regulations have reduced consumption of trans fat in recent years). According to an executive summary conducted by researchers from the US Department of Agriculture, the average American consumed 85 grams of fat per day, comprising 35 percent of their daily caloric intake. In the Women's Health Initiative (WHI) randomized clinical trial, reduction of total fat consumption from 37.8% energy to 24.3% energy (at 1 year) and 28.8% energy (at 6 years) had no effect on incidence of heart disease, stroke, or total cardiovascular disease over a mean of 8.1 years (Howard et al., 2006). This suggests that the role of dietary fat itself in cardiovascular disease may not be as important as generally thought. A major limitation of the WHI study is that vigorous exercise, enough to improve cardiorespiratory fitness, was not part of the intervention. There is evidence to suggest that exercise and/or cardiorespiratory fitness may be more important than diet for reducing cardiovascular disease risk (Heroux & Janssen, 2010; Huffman et al, 2012; Kouki et al, 2012).

In the Aerobics Center Longitudinal Study, high cardiorespiratory fitness in men and women was associated with low mortality risk regardless of diet quality (Heroux & Janssen, 2010). In the Dose Response to Exercise Training Study, metabolic syndrome risk was influenced more by cardiorespiratory fitness than by diet (Kouki et al, 2012). For example, prevalence of metabolic syndrome was more than twice as high in persons with the healthiest diet but with low cardiorespiratory fitness as compared to persons with the unhealthiest diet score but a high cardiorespiratory fitness level. Furthermore, in the Study of Targeted Risk Reduction Intervention through Defined Exercise, significant improvements resulting from exercise training were independent of diet quality (Huffman et al, 2012).

These studies are supported by a number of acute exercise studies demonstrating the beneficial effects of exercise in ameliorating the deleterious effects of a high-fat or high-sugar meal (Tyldum et al., 2009; Weiss, Arif, Villareal, Marzetti, & Holloszy, 2008; Padilla, Harris, Fly, Rink & Wallace, 2006). In a 2009 study, Tyldum and colleagues examined the effects of continuous moderate intensity exercise and high intensity interval exercise on endothelial function following a high fat meal. Before consuming the high-fat meal, researchers had the participants perform a single bout of exercise (continuous moderate intensity or high intensity interval) the day before. Approximately 16 hours after the exercise both the moderate and high intensity sessions improved endothelial function by 20%,($p < 0.01$) and by 45% ($p < 0.01$) respectively. However, after consuming the high fat meal, the high intensity interval exercise had not only prevented the normal decline in postprandial endothelial function, but brought about an increase in flow mediated dilation from baseline despite the high-fat meal-induced lipemia as compared to the continuous moderate intensity exercise and control groups. Results from this study found that the efficacy of exercise on mitigating negative effects was intensity dependent. Similarly, Weiss et al (2008) found that a single session of endurance exercise performed the day before consuming a high-sugar meal (candy bar and a sugar-sweetened soft drink) was sufficient to attenuate the impairment in endothelial function that occurred after ingestion of the high-sugar meal without exercise. In addition, the rise in postprandial plasma glucose and insulin concentrations was diminished, thus improving the insulin sensitivity index.

One unresolved issue is whether these acute exercise results can be extended to a more sustained exercise setting (e.g., over weeks) in which the diet is altered to increase

fat and sugar consumption on a daily basis. Short-term (i.e., 2 weeks) high-intensity exercise training that significantly improves cardiorespiratory fitness is effective in ameliorating the negative effects of postprandial hyperglycemia. In 2011, Little and colleagues examined the effects of six exercise sessions of ten one-minute intervals at 90% of maximal heart rate on blood glucose regulation. Following the interval exercise training protocol, there was a significant reduction in 24-hour glucose concentration from 7.6 ±1.0 to 6.6±0.7 mmol/l ($p<0.05$) as well as 3-hour postprandial combined area under the curve (AUC) for breakfast lunch and dinner (pre: 11,066±1,703 vs. post: 9,572±995 $mmol \cdot l^{-1} \cdot day^{-1}$, $p<0.02$).

Also, endothelial function improvements with exercise training appear to be maximized after just 2 to 4 weeks of training (Tinken et al, 2008). Because adverse effects of dietary changes can be observed within weeks (Silbernagel et al, 2011), it would be important to establish whether regular exercise could mitigate the anticipated deleterious effects on cardiovascular risk that could be expected during a short-term dietary intervention that increased fat and sugar consumption without overtly attempting to alter other dietary habits.

The mechanism for exercise related improvement on endothelial function despite conditions of lipemia may be the result of various mechanisms. Tyldum et al (2009) demonstrated that the improvement in endothelial function during the postprandial period was significantly correlated with total antioxidant capacity of the blood, with high-intensity interval exercise having the most potent effect. Though this explanation is merely one theory, this would suggest that the benefits maintain a link to NO bioavailability.

The connection between antioxidant status and NO bioavailability is supported by studies in which super-physiologic NO levels were induced by supplementing a high fat meal with sublingual nitroglycerin. In this case, there were no deleterious effects on endothelial-dependent vasodilation following the intake of a high fat meal (Xu et al., 2011). Counterevidence to this theory has been shown through other exercise studies that suggest that acute exercise increases the circulation of free radicals in the blood which would inhibit the activation of nitric oxide subsequently resulting in a decline of vascular reactivity (Benjma & Ji, 1999; Bailey et al., 2003; Bailey et al, 2007). Researchers in this particular study compared the improvement in endothelial function to that seen in studies where antioxidants prevented post-prandial endothelial dysfunction when given concurrently with a high fat meal (Plotnick, Corretti & Vogel, 1997).

In effort to further understand the mechanisms behind exercise induced improvements in endothelial function despite a high fat diet, Park et al (2012) conducted a study on rats evaluating a number of different markers associated with cardiovascular health risk. Three groups of rats were studied, differing in diet and exercise status: low-fat sedentary, high-fat sedentary, and high-fat running. The rats in the high-fat group were fed a diet comprised of 45% fat. After 10 weeks, the high-fat sedentary group had decreases in acetylcholine (ACh)-induced and flow-induced vasodilatations in isolated, pressurized coronary arterioles, heart phosphorylated endothelial nitric oxide synthase (p-eNOS/eNOS) protein, coronary arteriole leptin (*ob*) receptor protein, phosphorylated signal transducer and activator of transcription 3 (p-STAT3/STAT3) protein, and coronary arteriole superoxide dismutase 1 protein in comparison to the high-fat running group. Additionally, the high-fat sedentary group had increases in percentage body fat,

serum leptin, coronary arteriole suppressor of cytokine signaling 3 (SOCS3) protein, and coronary arteriole gp91phox protein. The results from this study suggest that there are a number of possible mechanisms responsible for the ability of exercise to attenuate or abolish any deleterious effects of a high-fat diet on cardiovascular health.

Unfortunately, there is a lack of studies in humans investigating the magnitude of effect that exercise may play in maintaining vascular endothelial function despite a sustained increase in fat and sugar consumption. The results of the few studies examining the ability of exercise to ameliorate or abolish the negative effects of a high-fat or high-sugar diet are equivocal, and the potential mechanisms remain largely unknown. The elevation in total antioxidant capacity in the blood as a result of a single exercise bout may explain the preservation of vascular function during a single postprandial setting, but no studies have been published to address whether this relationship extends to habitual diet conditions. Therefore, the objective of this dissertation research is to determine whether regular exercise can prevent deterioration in vascular function expected to occur as a result of an increased consumption of fat and sugar during a three-week period in college-aged men, and to determine whether this is related to changes in antioxidant capacity, nitric oxide availability, and other established blood biomarkers of cardiovascular risk.

Objective

The objective of the study is to determine if exercise training can prevent the anticipated deleterious effects of a fat-sugar supplemented diet (in the form of two doughnuts per day, 6 days per week, for 3 weeks) on endothelial function and blood markers of cardiovascular disease risk.

Hypotheses

H_1:

Fat-sugar supplemented diet will impair endothelial function and result in a worsened cardiovascular risk profile as assessed by fasting blood lipid profile, glucose, insulin, nitric oxide, total antioxidant capacity, and C-reactive protein.

H_2:

Exercise training will prevent deleterious effects of a fat-sugar supplemented diet on markers of cardiovascular risk.

Delimitations

This study is delimited to male participants between the ages of 18-30 that are generally sedentary, but otherwise metabolically healthy. There are no weight requirements for this study. Participants will be excluded from this study if they are currently involved in any form of regular aerobic exercise. Current dietary intake will not be considered for participant selection but will be required to remain consistent throughout the entirety of the study. Once enrolled in the study participants will not be permitted to participate in any form of aerobic activity outside of the protocol required for the experimental group to which they are assigned.

Assumptions

It is assumed that the participants will comply with the testing protocol outside of the lab. Following proper calibration protocols, the safety, proper use, and accuracy of all testing instruments is assumed. Honesty is assumed on the part of the participant to uphold the integrity of the study instructions to:

- Abstain from exercise greater than what is needed for performing activities of daily living (ADL) during the 3 weeks of testing.
- Refrain from making any alterations to their normal diet during the testing period.
- Come to the lab in a fasted state (10 hours or overnight) prior to testing day only consuming water.

Limitations

The number of subjects in our study will be small but sufficient to reach statistical power for the primary (endothelial function) and secondary (plasma lipids) measure. All participants will all be healthy college-age males, and it is not known whether the present training protocol will give similar adaptations in other populations such as women or subjects with cardiovascular disease. A large dietary fat-sugar load will be utilized in this investigation. Although meals with this amount of fat and sugar are frequently consumed, the effects of the consumption of three weeks of a fat-sugar supplemented diet may not be reflective of a consistently high fat-sugar diet. While it appears reasonable to assume that changes in vascular reactivity due to acute high-fat-sugar supplementation are transitory in nature, the longevity of the enhancement will not be measured in the current study. This study will not directly assess the effects of training on insulin sensitivity using hyperinsulinemic-euglycemic clamps and therefore cannot conclude whether low-volume HIT improves muscle insulin sensitivity with accuracy beyond the HOMA calculations.

Chapter 2

REVIEW OF LITERATURE

Cardiovascular disease (CVD) is the leading cause of death in the U.S., accounting for approximately 810,000 deaths annually (Johnson et al, 2009). Many risk factors for CVD such as diabetes, obesity, and smoking are associated with increased oxidative stress and inflammation, suggesting that these responses are involved in the initiation and pathogenesis of CVD. Because of the strong association between oxidative stress and vascular health, it is important to examine the mechanisms that regulate vascular endothelial function and define dietary strategies that can attenuate the oxidative stress responses that modulate these mechanisms.

A number of studies have shown that weight-related health conditions are significantly improved with just modest amounts of weight loss if they are brought about by increases in physical activity (NIH, 1998). Though over seventy percent of Americans have reported trying to lose weight at least once in the past 4 years, very few use the approach of moderate calorie reduction and an increase in physical activity. A meta-analysis examining the nutrient breakdown of popular diets did a comparison against the typical American diet. The typical American diet consists of 2200 total calories, 35 percent from fat, 50 percent from carbohydrates, and 15 percent from protein (Freedman, King, & Kennedy, 2001).

Contributing to the typical American diet is fast food, a 165 billion dollar industry in the US alone. The increased intake specifically in energy and saturated fat has been a large contributor to the increased risk of cardiovascular disease related to regular fast food consumption. In an effort to assess the effects of fast food consumption on vascular

function and markers of cardiovascular disease, Rudolph and colleagues (2012) evaluated items of various nutrient contents (burger and fries, vegetarian burger and fries, vegetarian burger and side salad) on healthy individuals. Results from this study showed a significant decrease in FMD after all meals but no significant different between meals. The results of this study show that there was no benefit to consuming the arguably healthier vegetarian burger or side options to the traditional beef burger and French fries in respect to vascular reactivity.

The western pattern diet (high intakes of red meat, processed eat, refined grains, french fries, and sweets/desserts) has been evaluated in comparison to a prudent pattern diet (high intakes of vegetables, fruit, legumes, fish, poultry, and whole grains) in a number of studies. Based on data from the Nurses' Health Study, researchers concluded that the western pattern diet was associated with a higher risk of morality from cardiovascular disease (22%), cancer (16%), and all-causes (21%) (Heidemann et al., 2009). What these results do not address is the role of cardiovascular fitness in mitigating these risks. Heroux and colleagues (2010) conducted an analysis of risk similar to the aforementioned review while controlling for self-reported physical activity levels. In agreement with the previous review these researchers identified the group at greatest mortality risk as the highest quintile of the Unhealthy Eating Index (characterized by a higher intake of red meat, added fat and simple carbohydrates, and a lower intake of non-citrus fruits). The 40% higher mortality risk associated with the lowest quintile of Unhealthy Eating Index was reduced by 55% after controlling for cardiorespiratory fitness. In fact, within the highest quintile of cardiorespiratory fitness, mortality risk was unrelated to Unhealthy Eating Index score. The implications of this

review suggest that cardiorespiratory fitness level, presumably achieved in large part through regular exercise, may offset much of the risk associated with unhealthy dietary patterns.

Diet vs. Exercise Debate

There is an ongoing debate as to whether exercise or nutrition plays a greater impact on protecting against cardiovascular disease risk. In 2010, Kouki and colleagues conducted a study aimed at examining the individual and combined effects of diet and exercise on the prevalence of metabolic syndrome. Dietary assessment was done via a four day food log specifically looking at intake of vegetables, fiber, and fish as well as the percentage of total energy consumption from saturated fatty acids. Cardiorespiratory fitness levels were assessed using a maximal cycle ergometer test. Results from the study indicated that individuals in the highest VO2max tertile and who met 3-4 dietary goals had the lowest prevalence of metabolic syndrome (5%) In contrast, individuals meeting the least number or dietary goals and scoring in the lowest tertile of fitness had the highest prevalence. The results also indicate that fitness levels are a stronger predictor of metabolic syndrome and not even a healthy diet can diminish the risk of low fitness levels. To examine this further Huffman et al (2012) conducted an exercise study in sedentary individuals while analyzing dietary patterns. After evaluating total fat, saturated fat, trans fatty acids, cholesterol, omega-3 fatty acids, and fiber researchers looked at the adjusted risk for heart disease. The results from this study revealed that independent of diet, exercise had beneficial effects on low density lipoprotein, high-density lipoprotein, cholesterol molecule size, and triglycerides ($P < 0.05$).

A review of the current literature as taken the diet versus exercise debate and looked into the development of coronary artery disease and vascular function (Taylor, 2012). In two similar studies, researchers established two cohorts of animals which were fed either a control or obesogenic high-fat diet for 10 weeks, and a third cohort that was allowed to voluntarily exercise by having access to running wheels as a 'primordial prevention' strategy, coinciding with the imposition of the high-fat dietary regimen (Booth & Lees, 2006; Park et al, 2012). In the sedentary group, high-fat feeding over 10 weeks in adult female mice produced a 4-fold increase in fat mass and in serum leptin concentration compared to low-fat controls, which was prevented by concomitant voluntary physical exercise; mice ran a remarkable 17.5 km per day on average. Diet-induced obesity was associated with profound impairment of endothelium-dependent relaxation to acetylcholine and flow-mediated dilatation in isolated coronary arterioles. The impairment in endothelium-dependent relaxation, which was nitric oxide dependent and associated with a reduction in eNOS phosphorylation, was entirely prevented by voluntary exercise and the absence of obesity despite on-going consumption of the high-fat diet. These results would appear to suggest that the presence of obesity rather than the high-fat diet *per se* is the predominant risk factor for coronary artery endothelial dysfunction.

Vascular Function and Markers of Cardiovascular Disease

The vascular endothelium is a thin layer of cells situated on top of the smooth muscle and lines the interior surface of the blood vessels located between cellular elements in the bloodstream and vascular smooth muscle cells in the blood vessel wall. The endothelium acts as a sort of biophysical sensor that holds the ability to respond to

changes in both the physical and metabolic environment to maintain vascular homeostasis. Its main purpose is to regulate the passage of substances from the bloodstream to the vascular wall.

The healthy endothelium is an important regulatory organ in maintaining cardiovascular homeostasis by controlling the balance between several opposing forces, including vasoconstriction and vasodilation, growth promotion and inhibition, pro-coagulation and anticoagulation, pro-inflammation and anti-inflammation, and oxidation and anti-oxidation; all of which, if unbalanced, would contribute to atherogenesis (Zabinski, Saelens, Stein, Hayden-Wade & Wilfley, 2003; Deforche, De Bourdeaudhuij & Tanghe, 2006; Nassis et al, 2005). Pioneering experiments by Furchgott and Zawadzki (1980) showed that the presence of an intact endothelium is essential for acetylcholine (ACh) to activate endothelium derived relaxing factors which allows for dilation of the arteries (Alexander, Landsman, Teutsch & Haffner, 2003).

Pathology of Endothelial Dysfunction

Impairments in normal functioning of the vascular endothelium have been shown to be an early indicator of atherosclerosis and future cardiovascular disease (CVD) risk (Zabinski, Saelens, Stein, Hayden-Wade & Wilfley, 2003). While alterations in endothelial function are multi-factorial, many mechanisms implicated in the pathogenesis of VED, such as formation of atherosclerotic plaques, are related to increased oxidative stress.

Vascular homeostasis is centrally regulated by vascular endothelial cells by modulating biological processes such as blood vessel formation, coagulation and fibrinolysis, vascular tone and inflammation (Deforche, De Bourdeaudhuij & Tanghe,

2006). Endothelial cells respond to both circulating factors such as hormones and mechanical changes due to blood flow. The mechanical forces that regulate endothelial cells include blood pressure, the hydrostatic forces of blood within the blood vessel; and shear stress, the dragging frictional force created by blood flow. The nature of shear stress plays an important role in the maintenance of proper vascular function and cardiovascular health (Deforche, De Bourdeaudhuij & Tanghe, 2006). In fact, laminar shear stress, generated by blood flowing in a stable laminar pattern, is generally regarded as atheroprotective whereas the development of atherosclerosis is largely attributed to turbulent blood flow (Nassis et al, 2005). Greater laminar shear stress induces the release of endothelium-derived relaxing factors such as nitric oxide and acetylcholine.

While endothelial dysfunction is most often thought of as an impairment of endothelium-dependent vasodilation, it most likely also includes impairments in other endothelial dependent functions. The nature of endothelial dysfunction resulting in attenuation of NO-mediated responses is unclear, however several likely mechanisms of endothelial dysfunction include: alterations in signal transduction, reduced availability of L-arginine, modification of the expression of eNOS, altered availability of cofactors for eNOS, imbalance between the production of endothelium-derived constricting and relaxing factors, increased destruction of NO by reactive oxygen species (ROS), intimal thickening as a diffusion barrier, and increased production of endothelium-dependent constricting factors (endothelin being the most prominent).

Endothelial dysfunction has been shown to be present in a wide range of vascular disorders, including atherosclerosis, type 2 diabetes mellitus, and hypertension. It is thought that disruption of the functional integrity of the vascular endothelium plays an

integral role in all stages of atherogenesis ranging from lesion initiation to plaque rupture. In a prospective study by Schachinger and colleagues (2000), 147 consecutive patients underwent coronary endothelial function testing in response to several stimuli (acetylcholine, cold pressor test, flow-mediated dilation) and subsequently were followed for 6.7 years. Patients who presented with endothelial dysfunction at the inception of the study had a significantly greater number of cardiovascular events. Obesity is associated with both endothelial dysfunction and increased risk of CVD in older adults; therefore it could be hypothesized that a similar relationship would be found between these variables in adolescents and young adults as well. While the mechanisms that lead to obesity-caused endothelial dysfunction remain unclear, it is thought that both insulin and a chronic pro-inflammatory state play a critical role.

High Fat Diets

The physiologic impact of dietary fat is well established. In one of the earlier studies, Straznicky and colleagues (1993) compared the effects of either a high-fat or low-fat diet on blood pressure, cardiovascular reactivity and sympathetic activity. In this case subjects were followed one of the two diets for 2 weeks. After this time, heart rate, mean arterial pressure, total plasma cholesterol and low-density lipoprotein levels fell significantly in the low-fat diet group as compared to the high fat-diet group. Since then, results from a number of studies in this area were summarized in a recent literature review assessing the effects of reducing or modifying dietary fat consumption on various measures of cardiovascular disease and acute cardiovascular incidence rates. This meta-analysis suggested that modification of dietary fat intake in favor of increasing unsaturated fats rather than an overall reduction of dietary fats. A compilation of the

clinical trials indicated that reducing saturated fat intake but not overall fat intake reduced risk of cardiovascular events by 14% (Hooper et al, 2012).

That dietary fat quality (type) rather than total amount is more important for a number of health outcomes is highlighted by studyshowing that the consumption of saturated fat, but not unsaturated fat, impairs the anti-inflammatory properties of high-density lipoproteins as well as endothelial function (Nicholls, 2006). After the consumption of an isocaloric meal containing either polyunsaturated or saturated fats, researchers measured the impact on high-density lipoprotein inhibitory activity by assessing the rate of expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Flow-mediated dilation decreased at 3 hours post saturated fat consumption ($P < 0.05$). Interestingly, there was no significant post prandial difference in either group at 6 hours. This suggests that post prandial changes in vascular reactivity may be acute.

Though the effect of varying fat-subgroups has been documented, it is necessary to evaluate the level of dietary modification necessary to make an notable impact on risk reduction. Astrup et al conducted a similar review of the effects of dietary modification on cardiovascular risk defining the necessary level of modification for a 2-3% reduction in risk consuming a Western diet as a replacement of 1% of energy from saturated fatty acids with polyunsaturated fatty acids (2010). In contrast, conflicting studies have shown that many of the specific fatty acid subgroups do not have a significant influence on overall measures of cardiovascular health (Hu, Manson & Willett 2001). Additional health discrepancies related to the type of fat ingested extends to inflammatory markers (Peairs, Rankin & Lee 2011). What remain in question are the optimal dose of specific

fatty acids and the ability of certain fats to attenuate or negate the physiologic risks associated with elevated saturated fat consumption. On the other hand, the effects of saturated fat intake on metabolic markers and cardiovascular reactivity are well documented.

Vascular reactivity as determined by endothelial function is strongly effected by modifiable and non-modifiable (age, smoking, blood pressure and metabolic abnormalities) risk factors. Of these factors, research has shown that dietary intake significantly influences metabolic abnormalities. In agreement with this association, test meal studies have consistently shown that the consumption of a high-fat meal has detrimental effects on postprandial vascular function (Vafeiadou et al, 2012). An early study by Vogel and colleagues (1997) took a closer look at the impact of diet on endothelial in healthy subjects. Subjects were randomized into either a no-fat or high-fat meal group. The high-fat group saw a detrimental change in triglycerides and flow-mediated dilation at 2 and 4 hours post feeding ($P < 0.05$). In addition to these changes, there was a correlation between the rise in triglycerides (2 hours) (but not low-density lipoproteins) and the decline in endothelial function. This opens up the possibility that post prandial triglyceride levels may be one factor driving the change in flow-mediated dilation.

There has been recent evidence to suggest that a high-fat meal may also have avenues of indirect impact on vascular reactivity. High levels of saturated fat and cholesterol may contribute to increases in blood pressure through the development of plaques on vessel walls, which result in the reduction of both their diameter and elasticity. Each of these measures is a critical element in evaluating endothelial function.

Jakulj and colleagues (2007) have shown that a high-fat meal may increase sensitivity to psychological stress in healthy adults. In response to either a high-fat or low-fat feeding, systolic and diastolic blood pressure and total peripheral resistance were measured acutely following a variety of psychological stress tests. Results showed a significant rise in each of the aforementioned measures from baseline in the low-fat group versus the high-fat group.

One suggested mechanism by which this decline in vascular function occurs is through a disruption of the normal production of superoxide anions in the vasculature (Ross, 1999). Superoxide anions are known to trigger vasoconstriction through a reaction with nitric oxide. This reaction results in the production of peroxynitrite, a strong oxidizing agent that damages endothelial function.

TNF-α, a proinflammatory cytokine has also been shown to induce an impairment of endothelium-dependent vasodilation in a variety of vascular beds by increasing oxidative stress and decreasing the release of nitric oxide (Boger, 2004). TNF- α may have a direct action on decreasing the amount of nitric oxide released and endothelium nitric oxide production. Further, it may directly activate NAD(P)H oxidase and increase reactive oxygen species production in vascular smooth muscle (Chen, Montagnani, Funahashi, Shimomura, Quon, 2003). TNF-α has a major role in adipose tissue and there is evidence of a three-fold increase in TNF-α mRNA protein and circulating levels in obese individuals. Winkler et al. (2002) showed that increased levels of TNF-α may be one of the linking factors in the insulin resistance and endothelial dysfunction relationship.

Adiponectin is a collagen-like plasma protein that is produced by the adipose tissue and that is abundant in the systemic circulation. In response to a high-fat diet, plasma concentrations of adiponectin are decreased. Adiponectin plays an important role in the regulation of insulin action, and has been shown to be positively correlated with insulin sensitivity. In addition to its effect on glucose metabolism, adiponectin appears to modulate endothelial function. Adiponectin has been shown to stimulate production of NO (and suppress adhesion molecule expression in vascular endothelial cells. In three recent clinical studies in adults, hypoadiponectinemia has been found to be directly correlated with endothelial function of the peripheral arteries (Ouchi et al, 2003; Shimabukuro et al, 2003; Tan et al, 2004).

Serum antioxidant levels have recently been examined as a possible underlying cause of the oxidative stress-related impairments on endothelial function. Following the ingestion of a high-fat meal, the antioxidant enzyme glutathione peroxidase, as well as the urinary excretion of 8-epi-prostaglandin $F_{2\alpha,}$ were measured to assess the impact of serum antioxidants (Tsai, Li, Lin, Chao & Chen, 2004). Though no changes were observed in high-sensitivity C-reactive protein and adhesion molecules, there was a decline in endothelial function. Endothelial dysfunction was brought about in response to augmented oxidative stress incurred by the depletion of serum antioxidant enzymes and increased excretion of oxidative modification products.

Research has also examined the mechanism behind the acute proatherogenic effect of back to back high-fat meal ingestions. Tushuizen and colleges (2006) measured flow-mediated dilation as well as serum cholesterol levels after feeding healthy subjects two high-fat meals. Following the second high-fat feeding researchers noted significant

impairment of flow-mediated dilation as well as increased oxidized low-density lipoprotein concentration. Participants experienced a moderate rise in glucose and triglycerides following the feedings which remained just within the normal range. Although this may not be of concern for healthy individuals, the results may have important implications for individuals predisposed to heart disease or type II diabetes.

High Sugar Diets

An increase in sugar intake between 1970 and 2005 of approximately 20 percent, largely in response to the availability of added sugars and sugar-sweetened beverages, has demanded a greater look into the impact of sugar ingestion on the risk of obesity and cardiovascular disease. Based on the 2005 US Dietary Guidelines, added sugars greatly exceeds discretionary calorie allowances regardless of energy needs. The chronic excess of sugar has been linked with several metabolic abnormalities, adverse health conditions, and lack of essential nutrients. Studies have suggested that factors such as meal composition, method of food preparation and physiological factors influence the impact of sugar on the body's glucose response and overall health (McDonald et al, 1995; Chen, Halter, Porte, 1987; Modghaddam, Vogt, Wolever, 2006). Sucrose and fructose tend to increase postprandial triglycerides and very low density lipoprotein levels, which may augment lipemiaalso appear to be common abnormalities in a diet high in added sugars (Chong, Fielding, Frayn, 2007; Teff et al, 2004). Inconsistent results on the effects of different monosaccharides on lipid levels suggest that more research is needed to investigate the mechanism of action (Silbernagel, 2011). Studies have shown that a high consumption of high-sugar foods and beverages and foods is associated with evidence of increased inflammation and oxidative stress (Lui et al, 2002; Price, Price, Reynolds,

2001; Scribner, Pawlak, Ludwig, 2007). Though the exact mechanism(s) by which sugar intake is associated with these negative factors is not known, the resultant elevated risk for cardiovascular disease has been well documented.

Overwhelming research indicates that oxidative stress induced by postprandial hyperglycemia, which may be induced by a high-sugar diet in healthy individuals has been shown to occur concomitantly with vascular endothelial dysfunction. While the exact mechanisms causing endothelial dysfunction remain unclear and incompletely explored, insulin resistance and the presence of proinflammatory markers are two likely candidates. Insulin resistance classically refers to an impairment of the degree to which a given quantity of insulin lowers plasma glucose. Data now show that insulin resistance is associated with endothelial dysfunction and insulin sensitivity is inversely proportional to the development of atherosclerosis. Petrie et al. (1996) showed a close positive relationship between insulin sensitivity and basal endothelial NO production. There is a well-established relationship between reactive oxygen species (ROS) and NO. Hyperglycemia is associated with the generation of ROS. NO has a direct effect on oxidative stress by scavenging ROS, and NO inactivation is enhanced in the presence of excess ROS. An overproduction of ROS may injure the endothelial cell membrane, inactivate NO, and cause oxidation of BH4.

Evidence exists to suggest that high-sugar or high-fat intake individually impairs endothelial function. Additional studies have examined the possible cumulative effect of hypertriglyceridemia and hyperglycemia on endothelial function (Ceriello et al, 2002). Participants underwent 3 feedings: a high-fat load, high-sugar load, and a combined load. Following the combined load there was a decrease in flow-mediated dilation and a rise in

nitrotyrosine that was more pronounced than either high-fat or high-sugar feedings alone. The rise in nitrotyrosine would support the theory that oxidative stress is the common mediator of endothelial dysfunction.

There is an established association between insulin and leptin as regulators of appetite and weight. Insulin is a trigger for leptin, both of which are transported to the central nervous system where they interact with neuropeptides in the hypothalamus that are known to impact food intake. Studies have shown a reduction in both insulin and leptin levels following the consumption of high-fat meals despite sufficient energy consumption (Havel et al, 1999; Romon, Lebel, Velly, Marecaux, Fruchart, Dallongeville, 1999). Because low levels of leptin tend to stimulate an increase in appetite, it could be suggested that high-fat and/or high-sugar diets could promote weight gain by leading to excessive energy intake.

Exercise and Cardiovascular Disease

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Acute vs. Chronic Adaptations to Exercise

Results from previous exercise studies suggest that chronic exercise results in improved endothelial function. Endothelium-dependent responses of the brachial artery in young healthy men were improved after just ten-weeks of regular physical exercise training at a moderate intensity (Clarkson et al., 1999). Another 12-week exercise program improved endothelial function in patients who had metabolic syndrome, but who were otherwise asymptomatic for cardiovascular disease (Lavrencic, Salobir, & Keber, 2000). Another recent study showed that endurance-trained men did not demonstrate an age-related decline in endothelium-dependent vasodilation (Tounian et al, 2001). The results in this study demonstrated that both middle aged and older men who regularly perform aerobic exercise exhibited greater acetylcholine-mediated vasodilation than their sedentary counterparts. Additionally, regular aerobic exercise restored the loss of endothelium-dependent vasodilation in previously sedentary middle aged and older men. In this case, patients with documented coronary artery disease showed an improvement in coronary endothelial function after just four weeks of vigorous exercise training. The latter results suggest an upregulation of the eNOS gene in response to chronic increases in shear stress and blood flow brought about by exercise.

In evaluating endothelial function, the time course of change has proven to be a key component in vascular adaptation. Studies show that approximately two to three weeks of training is optimal to identifying changes in endothelial function. This is in part due to the physical increase in vessel diameter that appears as a result of chronic exercise training. In 2008, Tinken et al theorized that short-term exercise training enhances eNOS and nitric oxide bioactivity and chronic exercise training induces arterial remodeling resulting in an increase in arterial size (Delp et al.1993; Sessa et al. 1994; Sun et al. 1994; Delp & Laughlin,1997; Brown, 2003; Prior et al. 2003). In effort to take a closer look at the chronological changes in vascular structure and function in response to exercise training their study examined conduit artery flow mediated dilation and conduit dilator capacity at two week intervals over the course of an 8 week training intervention. Results revealed increases in flow mediated dilation from baseline (5.9±0.5%) to 2 weeks (9.1±0.6, P <0.01) and 4 weeks (8.5±0.6%, P <0.01) but a return toward baseline at 6 and 8 weeks. In contrast, brachial artery dilation capacity progressively increased significantly (P <0.05) from baseline (8.1±0.4%) at weeks 2 (9.2±0.6), 4 (9.6±0.6%), 6 (10.0±0.5%), and 8 (10.5±0.8%). These results suggest that functional changes in vascular health preceded physical adaptations of arterial remodeling.

It is well accepted that chronic cardiovascular exercise can ameliorate the negative effects of an unhealthy diet. Additionally, it has also been established that a high-fat meal causes significant post-prandial impairment of endothelial function. With this additional knowledge, it is necessary to solidify the effects of a single bout of exercise on the acute physiologic response to a high fat meal. Padilla and colleges (2006) examined this question by conducting a 3-condition study (low-fat meal, high-fat meal,

high-fat meal + exercise) and measuring 4-hour post prandial flow-mediated dilation. FMD was significantly elevated above pre-prandial values following the high fat meal + exercise (5.61±1.54 to 8.72±0.94%, $P = 0.005$) but was unchanged following the LFM (6.17±0.94 to 7.18±1.31%, $P = 0.317$) and the HFM (5.73±1.23 to 4.29±1.64%, $P = 0.160$). These findings suggest that a single aerobic exercise session cannot only counteract the postprandial endothelial dysfunction induced by the ingestion of a high-fat meal, but also increase brachial artery FMD in apparently healthy adults.

In addition to acute changes in vascular reactivity in response to exercise training regardless of feeding scenarios, research also shows an improvement in post-prandial lipemia. In an acute exercise study utilizing sprint interval cycling, participants experienced 3 testing conditions – exercise with no energy replacement, exercise + energy replacement, and control (Freese et al, 2011). Participants performed exercise one evening either maintaining an energy deficit or feeding for energy balance before a 13-hour overnight fast. The following morning participants had baseline venous samples taken (insulin, glucose, cholesterol, triglycerides) and were fed a high-fat meal after which researchers monitored blood levels at 0, 30, 60, 120, 180 minutes. The postprandial area under the curve ($mmol \cdot l^{-1} \cdot 3h^{-1}$) triglyceride response was significantly lower in energy deficit (21%, $P < 0.006$) and energy balance groups (10%, $P < 0.044$) than in control, and significantly lower in energy deficit (12%, $P < 0.032$) than in the energy balance group. The energy deficit created by exercise plays an important role in the reduction in post prandial, suggesting that delaying replacement of the energy used during exercise has important health benefits. The results from this study support the growing literature indicating that the physiological effects and health benefits of high-

intensity, low-volume exercise are similar to those of moderate-intensity, high-volume exercise training.

High Intensity Interval Training

Increasing interest has risen in the benefits of high-intensity interval training in decreasing risk of cardiovascular disease. In 2008, Perry et al conducted study to identify the ability of high-intensity aerobic interval training to influence the carbohydrate and fat oxidation capacity in skeletal muscle as well as whole-body metabolic adaptations. Participants performed 10x4 minute intervals at 90% VO2peak separated by 2 minutes of active rest three times a week for 6 weeks. To examine changes in oxidation capacity researchers collected muscle biopsies at rest, after 5 minutes of a maximal exercise test, and at exhaustion. Adaptations during exercise included reduced glycogenolysis, lactate accumulation, and substrate phosphorylation (0–5 min of total expenditure), unchanged carbohydrate oxidation, and approximately 2-fold greater time to exhaustion; and increased fat oxidation at 60% of pre-training *V*O2 peak. This study demonstrated that 3 sessions per week of repeated high-intensity exercise over 6 weeks is an effect method to increase whole-body and skeletal muscle capacities to oxidize fat and carbohydrate in previously untrained individuals.

Sufficient evidence has long existed to support the efficacy of endurance training on incurring cardiac adaptation ultimately leading to a reduction of cardiovascular risk. The challenge that remains is the identification of the changes at the cellular level that beget these adaptations. In 2009, Wisloff and colleagues conducted a study to test whether a dose response effect of training on cardiac response exists in regards to training intensity. During training participants performed four, 4-minute intervals at 90-95%

VO2peak separated by three minutes of active rest. In comparison to participants who performed continuous exercise at 70% VO2peak, the high-intensity interval group made significant improvements in general cardiovascular health markers such as, left ventricular remodeling, ejection fraction and endothelial function as a result of training. These results suggest that improvements in cardiovascular function in response to exercise may be dose dependent as related to intensity.

Similar research studies have taken a different look at the benefits of interval training on metabolic indices related to metabolic syndrome. Little and colleagues (2011) also sought to determine if high-intensity training would have a comparable if not superior effects on hyperglycemia and mitochondrial capacity than commonly prescribed endurance training with despite reduced exercise volume. Participants in this study performed ten, 1-min intervals on a cycle ergometer at 90% HRmax separated by 1 minute of active rest three times per week for 2 weeks. Average 24-h blood glucose concentration was reduced after training (7.6±1.0 vs. 6.6±0.7mmol/l) as well as post-prandial area under the curve ($P<0.05$). Training also increased muscle mitochondrial capacity as evidenced by higher citrate synthase maximal activity (20%) and protein content of Complex II 70 kDa subunit (37%), Complex III Core 2 protein (51%), and Complex IV subunit IV (68%, all $P<0.05$). Mitofusin 2 (71%) and GLUT4 (369%) protein content were also higher after training (both $P<0.05$). Results show that low-volume, high-intensity training can effectively control blood glucose levels and improve metabolic capacity of skeletal muscle.

A recent study by Goto and colleagues (2003) examined the effects of different intensities of exercise on endothelium-dependent vasodilation in humans. Subjects (26

healthy males) were randomly assigned to one of three training groups: mild intensity (25 percent VO2 max), moderate intensity (50 percent VO2 max), or high intensity (75 percent of VO2 max) (2003). Results showed that twelve weeks of moderate-intensity exercise, but not mild- or high-intensity exercise, significantly increased acetylcholine-induced vasodilation of the brachial artery. Both 8-hydroxy-2'-deoxyguanosine and serum concentrations of mallondialdehyde-modified low-density lipoprotein were measured to evaluate the amount of oxidative stress present in each group. Both indices of oxidative stress were increased in the high-intensity group after exercise, whereas in the moderate-intensity group both were decreased after the exercise training. Those subjects that trained at a low-intensity had neither increased oxidative stress markers nor any improvements in endothelial function. These findings suggest that moderate-intensity aerobic exercise successfully augments endothelium-dependent vasodilation, while high-intensity exercise increases oxidative stress that may interfere with an increased production of nitric oxide.

Interaction of Diet and Exercise

When examining the effects of exercise on physiologic markers of health it is necessary to also consider the interaction effects of dietary intake. Though research has provided some insight into contributing factors such as impaired endothelial function and reduced flow-mediated vasoactivity, the exact physiological mechanisms responsible for the effect of dietary feedings on cardiovascular function are still largely unknown (Tsai, Li, Lin, Chao, & Chen, 2004; Vogel, Corretti, & Plotnick, 1997). Earlier animal studies provided some preliminary data on these changes. In a study looking at mice exposed to high-fat or low-fat feedings over the course of twelve weeks, researchers examined the

effects of exercise on metabolic indices (Duggan, Hittel, Sensen, Weljie, Vogel, Shearer, 2011). Each feeding group was further divided into exercise or non-exercise conditions. After analyzing metabolites significantly changing in response to the high-fat feeding, results showed that exercise was able to mitigate most but not all of the negative effects of the high-fat diet.

In effort to bridge the knowledge gap to human subjects, Faulk & Bartholomew aimed to reexamine the effects of consuming a high-fat meal on cardiovascular reactivity while controlling for protein content and to determine if acute exercise can moderate the impact of dietary fat consumption on cardiovascular reactivity (2012). Participants were grouped into either high- or low-fat diets and further into exercise or no exercise. Results from this study indicated that consumption of a single high-fat meal may lead to increases in mean arterial pressure, however a single bout of high-intensity exercise can mitigate rises in both mean arterial pressure and heart rate reactivity.

In 2009, Tyldum and colleagues took a closer look at the effects of both exercise and high-fat meals on endothelial function. Brachial artery flow-mediated dilation was assessed in healthy men before and after a high fat meal after one of three conditions: an extended rest, a single bout of continuous moderate-intensity exercise, and high-intensity interval exercise. Prior to consuming the high-fat meal, researchers found an improvement in flow-mediated dilation in the continuous moderate-intensity exercise (+20%, $p<0.01$) and the high-intensity interval exercise (+45%, $p<0.01$). After this exercise session participants consumed the high-fat meal that had previously been shown to impair flow-mediated dilation at baseline. Post prandial endothelial function was assessed at 30, 120, and 240 minutes and compared to baseline. Across these time points,

exercise was able to mitigate the decline in flow-mediated dilation. In contrast to the continuous and control group the high-intensity group exhibited a sustained elevation in endothelial function despite postprandial lipemia.

Diets specifically high in saturated fatty acids have been shown to have deleterious effects on metabolic markers and increase cardiovascular disease risk in otherwise healthy individuals. In response to these previous findings, a recent study has looked at the ability of aerobic exercise to mitigate these effects (Oretga et al, 2013). All participants in this study increased saturated fat intake from 31±10 to 52±14 grams per day for two weeks while maintaining total fat intake. One-half of the participants also underwent 11 cycle-ergometer sessions of 55 minutes at 60% of VO2peak, while the other half remained sedentary. As compared to baseline, there were no changes in body weight, composition, plasma free fatty acids, and insulin sensitivity. In contrast, the researchers did find an increase in total cholesterol (147±8 to 161±9 mg/dL^{-1}, P =0.018) and low-density (71±10 to 82±10 mg/dL^{-1}, P = 0.034) in the diet only group.

At the cellular level it is uncertain as to whether increasing dietary fat availability after exercise alters the exercise-induced increase in insulin sensitivity. Several studies have also associated insulin resistance with an accumulation of triglycerides within the muscle cell (Goodpaster, Thaete, Simoneau, and Kelly, 1997; Oakes et al, 1997; Pan et al, 1997), but the direct effect of IMTG concentration of insulin sensitivity remains unknown (Furler et al, 2001; Hegarty, Fuler, Ye, Coone, & Kraegen, 2003). Researchers investigated whether adding fat calories to meals after exercise alters glucose tolerance the next day (Fox, Kaufman & Horowitz, 2004). Healthy men cycled 90 min at 66±2% peak oxygen uptake followed by a maximum of five high-intensity intervals. Following

the exercise, participants ingested three meals containing either low-fat (5% energy from fat) or high-fat (45% energy from fat) foods. Muscle biopsies taken 24 hours after exercise revealed an increase (~20%) in intramuscular triglycerides in the high-fat group. Regardless of the rise in intramuscular triglycerides, there was no difference in glucose tolerance between groups.

Mixed findings regarding the relationship between carbohydrate metabolism and lipid levels have driven efforts to identify the mechanisms at the cellular level that may explain physiological responses to high fat feedings. To accomplish this, more recent research has focused on intramyocellular lipids. While consuming both a high-fat and low-fat feeding during two separate 5 week periods during a crossover training study, healthy subjects performed time trials on a cycle ergometer as well as a completing a half marathon (Vogt et al, 2003). Despite a significant ~2-fold rise in intramyocellular lipids during the high-fat feeding period, muscle glycogen stores were maintained. Findings of these two studies suggest the inverse relationship between intramyocellular concentration and insulin sensitivity may only exist in sedentary subjects (Goodpaster et al, 1997; Oakes et al, 1997).

As discussed in previous studies, responses to high-fat feedings may vary by body composition as well as activity level. In young, metabolically healthy males, researchers examined the effect of prior exercise on postprandial triglycerides, insulin, glucose, and inflammatory markers (MacEneaney et al, 2009). Participants were grouped by body mass index. On two different occasions each participant underwent a 6 hour oral fat tolerance test following either an exercise bout or in a rested state. Exercise was shown to reduce postprandial triglyceride area under the curve by 20 percent ($p<0.01$). There

were no significant changes in glucose, insulin or inflammatory markers between normal weight and overweight participants.

To better evaluate the effect of exercise on postprandial markers of glucose and insulin, it is necessary to consume a comparable load of glucose to that which was given in the high-fat trials. Weiss and colleagues (2008) sought to determine whether endurance exercise performed 17 hours before high-sugar ingestion attenuates postprandial impairment of endothelial function. Data for flow-mediated dilation, glucose, insulin, and thiobarbituric acid-reactive substances, a marker of oxidative stress, were collected from all participants to assess change from before to after the ingestion of a candy bar and a soda. In both rested and exercised states, there was a significant decrease in flow-mediated dilation following high-sugar ingestion, however there was a significant shift creating a greater area under the curve in the previously exercised group ($P = 0.01$). Prior exercise brought about similar improvements in the areas under the curve for both glucose ($P = 0.05$) and insulin ($P = 0.0007$) as well as significant improvements in insulin sensitivity index (10.8 ± 0.7, $P = 0.01$). Results support the efficacy of exercise in glucogregulation independent of improvements in oxidative stress.

In a trial looking at the aforementioned metabolic markers, Strohacker et al (2012) also evaluated the effect of a single pre-meal exercise bout on endothelial stress and inflammation following a high-fat meal. Participants completed two trials, one resting and one moderate-intensity cycling bout prior to a high-fat feeding. In agreement with previous research, Strohacker and colleagues observed no significant impact of premeal exercise on postprandial concentrations of triglycerides, total cholesterol, HDL cholesterol or glucose (MacEneaney et al, 2009). Evaluation of the changes in monocyte

cell surface receptor expression as well as endothelial microparticles between groups revealed a significant group difference. Moderate-intensity, premeal cycling successfully mitigated postprandial increases in the classic subset of monocyte cell surface particles CD18 ($p=0.001$) and CD11a ($p=0.026$). Similarly, endothelial microparticle count was 47% greater 3 hours post feeding in the resting, no-exercise condition ($p\leq0.05$) than the pre-meal cycling condition. Receptor expression and peripheral blood monocytes as those measured in this study may play prominent roles in inflammation and atherosclerosis.

It has been established that in states of hyperlipidemia, oxidative stress, and vascular inflammation, monocytes affix to the endothelial lining which subsequently leads to macrophage accumulation and foam cell formation – a precursor to atherosclerosis. The current pool of research has highlighted the need to further identify and understand the mechanisms by which exercise acts as a moderator of cardiovascular health. The extrapolation of the findings in the aforementioned research to vascular function and markers of metabolic health is potentially critical in supporting the efficacy of exercise for the prevention of cardiovascular disease regardless of dietary intake.

Chapter 3

METHODS

Experimental Subjects

Twenty-one (21) male subjects between the ages of 18 - 30 years old were recruited from Phoenix and surrounding areas for participation in this study (see Appendix D). Eligible participants were generally sedentary (exercising less than 1 day per week), were in generally good health, had no restrictions for participating in vigorous intensity physical activity, and were not taking any medications for blood pressure, cholesterol, diabetes or a heart condition. Volunteers were excluded from the study if they had a history of smoking, cardiovascular disease, renal or liver disease, were taking hypoglycemic and / or hypertensive medication, had been physically active for the 3 months, or had any additional conditions that would be contraindicative of exercise. The Institutional Review Board of the Arizona State University approved all methods and procedures.

Study Design

All participants who responded to the recruitment flyer were provided a copy of the consent form to read, and any questions they had about the study were answered by study personnel. All aspects of the study were explained and written consent was obtained. Participants filled out a physical activity readiness questionnaire (PAR-Q) to ensure that they were suitable candidates for enrollment. If they answered “yes” to any of the questions, they were not allowed to participate. If they answered “no” to each question, they were allowed to participate. Those who decided to participate after

signing this consent form underwent a flow-mediated dilation (FMD) procedure (see below) to make sure a viable acoustic window of their brachial artery could be obtained.

Participants who agreed to take part in the study reported to the Healthy Lifestyles Research Center in ISTB3 on the ASU Polytechnic Campus for all visits. Participants randomly assigned to the doughnut-supplemented group were required to have 11 additional visits beyond the screening visit (visits 2 through 12) over a 3-week period to pick up their doughnuts. Participants randomly assigned to the doughnut-supplemented + exercise group participated in a total of 14 visits beyond the screening visit (visits 2 thorough 15) over a 3-week period. Visits are described in detail below.

Baseline Testing

The participants reported for baseline testing at the ISTB3 building following an overnight fast (nothing but water after 10 PM). Participants first had a measurement of brachial artery flow-mediated dilation taken, followed by resting blood pressure measurement. After two blood pressure readings, participants then had body composition assessed (BOD POD). Following this measurement, participants had a fasting blood draw. For the final test, all participants performed a VO_2max test on a cycle ergometer to determine maximal oxygen uptake. Participants were randomly assigned to either the doughnut-supplemented + exercise group or doughnut-only group.

Visits 3 through11 (Doughnut-Supplemented Group)

These visits were designated for picking up the doughnuts that were provided to each participant during the 3-week study. Participants picked up bags of 4 fresh doughnuts each at the ISTB3 laboratory on Monday, Wednesday and Friday of each week.

Visits 3 through14 (Doughnut-Supplemented + Exercise Training Group)

The participants in the doughnut-supplemented + exercise training group reported to the ISTB3 building for exercise training. Participants trained four (4) days per week for three (3) consecutive weeks. Training sessions were not required to occur on consecutive days. All exercise training were performed on either a motor-driven treadmill (continuous exercise sessions) or a cycle ergometer (interval and/or continuous exercise sessions). The aerobic exercise training protocol consisted of 2 sessions/week of continuous exercise and 2 sessions/week of interval exercise. The exercise protocols were as follows:

- 2 days per week of continuous exercise: 30 minutes at a heart rate associated with 75% maximum heart rate determined from the baseline cycle test.
- 2 days per week of high-intensity interval exercise:
    - One day per week: Ten 1-minute intervals at 90-95% of maximum heart rate, separated by 1 minute of active recovery at 60% of maximum heart rate (Little et al, 2011).
    - One day per week: Four 4-minute intervals at 90-95% of maximum heart rate, separated by 3 minutes of active recovery at 60% of maximum heart rate (Wisloff et al, 2009).

Each exercise session was preceded by a 5 minute warm-up (50-60% of maximum heart rate), and concluded with a 5-minute cool-down (50-60% of maximum heart rate).

Final Visit (Visit 12 for Doughnut Only Group; Visit 15 for Donut-Supplemented + Exercise Training Group)

Approximately 48 hours following the last training session, the participant reported to the ISTB3 building following a 10 hour (overnight) fast. Post-test appointments were scheduled within 1 hour of time of day of baseline testing to avoid error due to diurnal variation of some of the measurements. Participants first had brachial artery flow-mediated dilation measured, followed by 2 measurements of resting blood pressure. After two blood pressure readings, participants then had body composition assessed (BOD POD). Following this measurement, participants had a fasting blood draw. All participants finished testing with a VO_2max test on a treadmill to determine maximal oxygen uptake.

Measurement

Brachial Artery Flow Mediated Dilation (BAFMD)

This is a non-invasive assessment of the ability of the brachial (upper arm) artery to dilate in response to an increase in blood flow, and is assessed using ultrasound. This procedure is performed while the subject is lying supine on a padded ultrasound table. All measurements are made on the non-dominant arm. A blood pressure cuff is positioned on the subject's forearm, midway between the elbow and wrist. After recording baseline ultrasound measures on the upper arm, the blood pressure cuff is inflated to 250 mmHg for 5 minutes. The cuff is then deflated rapidly and brachial artery blood flow and arterial diameter are measured continuously for 5 minutes using the ultrasound probe. Ultrasound images were acquired using a Terason t3000CV ultrasound system (Terason Ultrasound, Burlington, MA) equipped with an 11-3L linear array transducer. FMD is calculated as the percentage increase in diameter from baseline to peak diameter, where baseline diameter was the average diastolic diameter over the 2-

min baseline, and peak diameter was recorded as the 10-s average of the highest diastolic diameter after cuff deflation. These procedures conform to the published guidelines of the International Brachial Artery Reactivity Task Force (Corretti et al, 2002).

Blood Draw

Subjects were asked to report for each blood draw (visits 2 and 12 for doughnut only group; visits 2 and 15 for doughnut + exercise training group) in the morning following an overnight fast (i.e., nothing but water after 10 PM). Intravenous blood was drawn via one of the common antecubital fossa veins (cephalic, median cubital, or basilic vein) for measurement of blood lipids, glucose, insulin, and antioxidant capacity. All blood draws were performed by a certified phlebotomist. Approximately 15ml (3tsp) of blood were collected for each blood draw. Blood will be analyzed for plasma lipids, glucose, insulin, C-reactive protein, nitric oxide availability, and total antioxidant capacity. Plasma was isolated and stored at -80 °C for later analysis.

Glucose was analyzed by using the glucose oxidase method. Serum lipids were assessed by using a cholesterol fluorometric assay kit (Cayman Item No. 10007640). The assay is based on an enzyme-coupled reaction that detects both free cholesterol and cholesterol esters. Nitric Oxide was analyzed by using a nitrate/nitrite flurometric assay (Cayman Item No.780051). This assay measures total nitrate/nitrite concentration by converting nitrate to nitrite and then measuring the fluorescence of 1(H)-naphthotriazole. Antioxidant capacity was assessed through a serum assay (Cayman Item No. 709001). The capacity of the antioxidants in the samples to prevent ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) oxidation was compared with that of Trolox, a water-soluble tocopherol analogue, and was quantified as millimolar Trolox equivalents. High-

sensitivity C-reactive protein was analyzed from serum samples using a sandwich ELISA, HRP-lablled antibody assay. All samples were analyzed in duplicate, and all samples for each subject were batch analyzed to eliminate interassay variability. Insulin will be analyzed using a double antibody radioimmunoassay. The homeostasis model assessment of insulin sensitivity (HOMA) will be calculated as follows (Matthews et al,1985; Chen et al, 2005):

HOMA = fasting glucose(mg/dL) * fasting insulin(μU/mL)/405

Blood Pressure

Resting blood pressure was measured using an automated blood pressure machine. The patient was comfortably seated in a chair and the deflated cuff applied to the right arm just proximal to the elbow with the bladder centered over the brachial artery. Proper cuff size was determined based on the standard recommendation that the bladder should be approximately 40% of the circumference of the limb tested (Brzezinski, 1990). Pressure was then rapidly increased to at least 30 mmHg higher than that which eliminates a palpable radial pulse.

Anthropometrics

Subjects' weight was measured on a standard Beam scale. Height was assessed on a stadiometer.

Body Composition

Body composition was measured using a Bod Pod (air displacement plethysmography). All participants were required to wear a swimsuit and swim cap during the BodPod test to minimize errors caused by air trapped in clothing and hair. Subjects were weighed prior to entering the BodPod, before sitting comfortably in the

measurement chamber. The door was then closed and the run begins. Each subject run took less than one minute. If the two subject measurements did not agree, a third run was done. The two closest runs were then averaged. Lung volume was estimated for each participant. This completes the measurement procedure, and results for %fat and fat-free mass are displayed.

VO_2max test

Subjects were equipped with a mask attached to flexible, low-resistance tubing, and Polar heart rate monitor for the metabolic measurement device (Parvo Truemax 2400TM) to measure ventilation and respiratory gas exchange data and heart rate continuously. After collecting resting data for 2 minutes, subjects pedaled on a stationary cycle ergometer at a cadence of their choice at 50 watts for 5 minutes for the warm-up phase. After the warm-up phase, load was increased continuously by 30 watts/minutes every minute until the subject was unable to continue. Verbal encouragement was given to all subjects throughout the entire test. The highest oxygen uptake during the test was taken as the value for peak VO_2.

Fat-Sugar Dietary Supplement

Each participant was required to consume 2 doughnuts per day, 6 days per week, for 3 weeks. Doughnuts were purchased by the study researchers at a local Dunkin Donuts on Monday, Wednesday and Friday morning of each week. Participants were required to come to the ISTB3 laboratory on Monday, Wednesday, and Friday of each week for doughnut pickup. This could be part of the regular exercise training visits for those in the exercise training group. On each occasion participants were given 4 doughnuts to consume over the following two days. This schedule was designed to

optimize compliance while ensuring that the doughnuts are fresh. Participants were provided a menu of doughnut options so that each participant would be able to choose the types of doughnuts that he would like most to consume. The menu was divided into three categories by energy content (250-299 kcal, 300-349 kcal, 350-399 kcal). Participants were instructed to select 4 doughnuts from each category each week (totaling 12 per week) to minimize variance in consumption between participants. All doughnuts contain between 260 and 400 calories, of which at least 70% of total calories were fat and sugar.

Food Frequency Questionnaire (ASA24)

The participants were required to complete the ASA24 recall at the beginning of the study to determine if dietary intake was homogeneous between groups at baseline. The ASA24 allows study participants to report their intake for the previous day from midnight to midnight. The Respondent application guides the participant through the completion of a 24hour recall for the previous day from midnight to midnight using a dynamic user interface. This application provides an animated guide to instruct participants and enhance use in low-literacy populations (with options to turn off the guide and/or the audio). This program asked respondents to report eating occasion and time of consumption and includes optional modules to query about where meals were eaten, who the respondent ate with, and television or computer use during meals. Respondents were provided a meal-based "quick list" of foods and drinks consumed the previous day and were able to find foods or drinks to report by browsing food groups or searching from a list of food terms derived from USDA's AMPM. This program guided respondents through detailed questions about food preparation and additions to assigned food codes from USDA's Food and Nutrient Database for Dietary Studies (FNDDS).

This program also uses images to assist respondents in reporting accurate portion size and allows the respondent to add or modify food and drink choices at multiple times. The report developed form the information provided includes a final review of the day's intake for each participant.

Daily Dietary Requirements

Each participant was encouraged to maintain his normal diet as reported to the ASA24 diet recall during the three weeks, other than consuming the 2 doughnuts per day provided by study researchers as a requirement for participating in the study.

Statistics

Data were assessed to first determine normality status of testing groups in this randomized control-group pretest-posttest design. Pre- and post-test comparisons were made, assuming no outliers and equal variances (Levene test), on blood pressure, blood lipids, glucose, insulin, insulin sensitivity, nitric oxide, hsCRP, total antioxidant capacity, and endothelial function. Statistical analyses were performed using SPSS 13.0 for Windows. Results are presented as the mean ± standard deviation with significance shown when $p<.05$. An independent t-test was conducted to compare the delta values for each variable between the control and exercise groups. An a priori power analysis was performed to determine the sample size necessary to detect significant changes in flow-mediated dilation of the brachial artery. It was determined that for a randomized control-group pretest-posttest, in order to detect a large effect size (Cohen $f = 0.5$) (Cohen, 1988) in brachial artery flow mediated dilation with $\alpha = 0.05$ significance level and power of >0.80 that sixteen (n=16) subjects would be needed to obtain statistical significance for this study (Tyldum et al, 2009). Accounting for a 20 percent dropout rate, twenty (21)

subjects were recruited for this study. Power calculations were performed using G*power 3.0 software (Faul, Erdfelder, Lang, Buchner 2007).

Chapter 4

RESULTS

Subject Characteristics

A total of twenty-one (n=21) college-aged, males were recruited for this study. Participants range in age from 18 to 30 years (24 ± 3.4) and consisted of 60 percent Caucasian, 20 percent Hispanic, and 20 percent African American. During the testing period, two subjects, one from each treatment group, were unable to complete the trial (one due to a scheduling issue and another due to a lack of transportation). Thus, all data represent a total of 19 individuals. No differences in age or BMI were found between groups at baseline. There were also no significant differences in age, blood pressure, body fat percentage, or VO_2peak (Table 1).

Dietary Intake

Based upon 24-hour dietary recall data there were no significant differences in baseline total kcal intake between the doughnut + exercise group and the doughnut only group (2499 ± 314 kcal/day) (p>0.05) (Table 2). The two groups did not differ with regard to macronutrient intake based upon the results from the ASA24 24 hour recall questionnaire.

All subjects successfully picked up their doughnuts, three times per week, during the study. The total energy content of the three dozen doughnuts did not differ between doughnut + exercise (11,640 ± 225 kcal) and doughnut only (11,696 ± 134 kcal) groups. Additionally, total fat (688 ± 39 g vs. 679 ± 28 g), saturated fat (311 ± 19g vs. 305 ± 17g), and sugar (153 ± 28 g vs. 145 ± 44 g) content of the three dozen doughnuts consumed did not differ between the doughnut + exercise and doughnut only groups

Table 1

Subject Demographics

Measure	Baseline Measures Doughnut +Exercise (n=10)	Doughnut Only (n=9)	Combined (N=19)
Age (years)	23.1±2.42	25.0±4.15	24.0±3.39
Weight (kg)	74.29±7.48	78.84±21.76	76.44±15.62
Height (cm)	176.03±5.76	176.88±6.00	176.43±5.72
BMI (kg/m^2)	24.03±2.79	25.04±6.15	24.51±4.58
Body Fat (%)	17.4±8.56	22.96±13.05	20.01±10.92
Blood Pressure			
Systolic	122.0±7.98	124.00±7.63	122.94±7.67
Diastolic	76.7±8.52	75.88±9.86	76.31±8.93
VO_2peak (L/min)	3.08±0.29	2.65±0.42	2.88±.414

Results are reported as Mean ± Standard Deviation.
Significance is set at p=0.05.

Table 2

Dietary Intake

Measure	Baseline Measures: Doughnut +Exercise (n=10)	Baseline Measures: Doughnut Only (n=9)	Baseline Measures: Combined (N=19)
Daily Intake			
Calories	2455±348	2499±314	2476±324
Fat (g)	76±11	78±9	77±10
Carbohydrates (g)	331±47	337±42	334±43
Protein (g)	110±16	113±14	111±14
Total Donut Intake			
Calories	11640±225	11697±134	11667±185
Fat (g)	688±39	680±28	684±33
Saturated Fat (g)	311±19	305±17	308±18
Sugar (g)	536±46	528±41	532±42
Daily Donut Intake			
Calories	645±13	650±7	647±11
Fat (g)	38±2	38±2	38±2
Saturated Fat (g)	17±1	17±1	17±1
Sugar (g)	30±2	30±2	30±2

Results are reported as Mean ± Standard Deviation.
Significance is set at p=0.05.

($p>0.05$).Daily caloric intake from the donuts was not significantly different between the doughnut + exercise group (645 ± 13kcals) and the doughnut only group (650 ± 7kcals). There were also no significant differences in average daily intake of total fat, saturated fat, and sugar ($p>0.05$).

Aerobic Fitness and Body Composition Changes

Subjects in the doughnut + exercise training group significantly increased VO_2peak after three weeks of training by ~7.8% (3.08 ± 0.29 L/min to 3.32 ± 0.29 L/min), whereas the doughnut only group showed no change in aerobic capacity (2.65 ± 0.42 L/min to 2.63 ± 0.44 L/min) ($p = 0.005$ between groups) (Table 3). Subjects in the exercise training group completed all 12 supervised exercise sessions, accounting for 100% adherence. Based on baseline VO_2peak and assigned training intensity, mean energy expenditure during exercise sessions was estimated to be 354 ± 22kcal per session at the start of training. Due to the observed increase in VO_2peak (~7.8%; or about 2.6% per week), the total estimated energy expenditure of the 12 training sessions was approximately 4,360 kcal per subject. This corresponds to approximately 38% of the energy value of the three dozen doughnuts consumed over the course of the study.

Changes in body weight, fat mass, and percentage body fat were all different between groups (Table 3). Whereas body weight remained unchanged in the doughnut + exercise training group (Pre = 74.3 ± 7.5 kg; Post = 74.2 ± 8.9 kg), body weight increased in the doughnut only group (Pre = 78.8 ± 21.7 kg; Post = 80.5 ± 21.5 kg) ($p = 0.036$ for group differences) (Figure 1). In the doughnut + exercise training group fat mass (Pre = 13.6 ± 7.8 kg; Post = 13.4 ± 7.6 kg) and percentage body fat (Pre = 17.4 ± 8.6 %; Post =

Table 3

Cardiovascular Fitness & Body Composition

	Doughnut + Exercise (n=9)			Doughnut (n=9)			
Measure	Pre	Post	Δ	Pre	Post	Δ	*p* value of Interaction
Body Weight (kg)	74.3±7.5	74.2±8.9	-0.1±1.3	78.8±21.7	80.5±21.5	1.7±2.0	p=0.036
Fat Mass (kg)*	13.6±7.8	13.4±7.6	-0.2±1.2	21.1±18.27	23.6±18.9	2.5±1.6	p=0.013
Body Fat (%)*	17.4±8.6	17.1±8.3	-0.2±1.4	22.9±13.1	24.4±12.7	1.5±1.1	p=0.014
VO2peak (L/min)	3.08±0.29	3.32±0.24	0.23±0.21	2.66±0.42	2.64±0.44	-0.02±0.105	p=0.005

Results are reported as Mean ± Standard Deviation.
*(n=8) in the Exercise Group

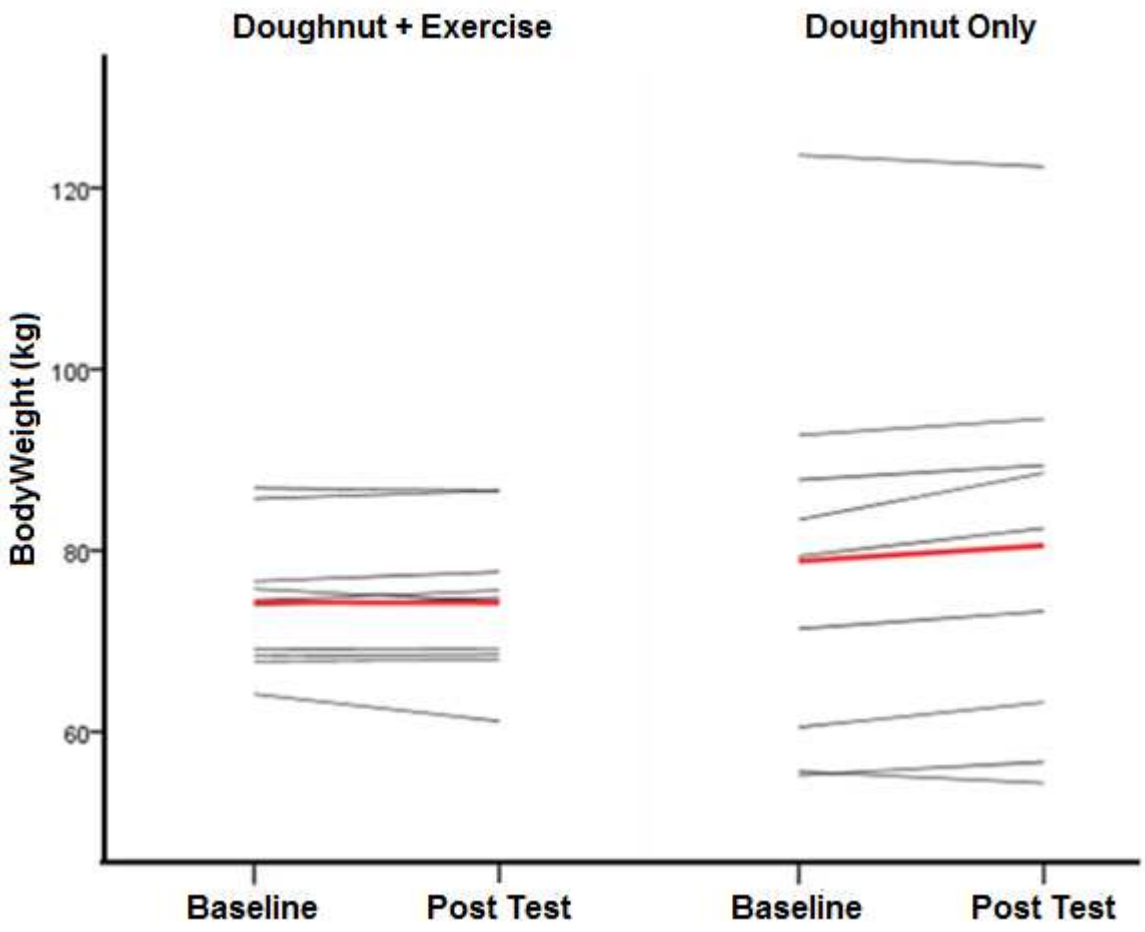

*Figure 1.*Change in Body Weight for the doughnut + exercise group versus the doughnut only group between baseline and post testing. Bolded line represents the group means for the doughnut + exercise (-0.05±1.2kg) and doughnut only (1.7±2.0kg). There was a significant interaction effect for body weight (p=0.036).

17.1 ± 8.3 %) did not change, in contrast to the doughnut only group which increased both fat mass (Pre = 21.2 ± 18.3 %; Post = 23.6 ± 18.9 %) (p = 0.013 between groups) and percent body fat (Pre = 22.9 ± 13.1 %; Post = 24.4 ± 12.7 %) (p = 0.014 between groups) (Figure 2).

Endothelial Function and Markers of Oxidative stress

Baseline and post-test values for endothelial function, baseline diameter, and peak diameter are shown in Table 4. There were no significant differences in blood pressure or flow mediated dilation from baseline to post testing between the doughnut + exercise and doughnut groups. There was a significant difference between the change in baseline diameter pre to post in the doughnut + exercise group (0.39±0.03 cm to 0.42±0.03 cm) (p=0.001) versus the doughnut only group (0.37±0.04 cm to 0.38±0.03 cm). Additionally there was a significant difference in peak diameter in the doughnut + exercise group (0.41±0.03 cm to 0.43±0.03cm) (p=0.04) but not in the doughnut only group (0.39±0.05 cm to 0.41±0.05 cm). Results for high sensitivity C-reactive protein (hsCRP), nitric oxide (NO), and antioxidants (AOX) are shown in Table 5. There was a significant change in nitric oxide availability between groups (-4.29 ± 3.39 uM vs. +4.33 ± 8.76 uM, p=0.014) in the doughnut only and doughnut + exercise groups respectively. There were no significant differences in C-reactive protein or total antioxidant capacity between treatment groups.

Serum Lipids & Blood Glucose

Baseline and posttest values for serum lipids (total cholesterol (CHOL), low-density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG)), fasting

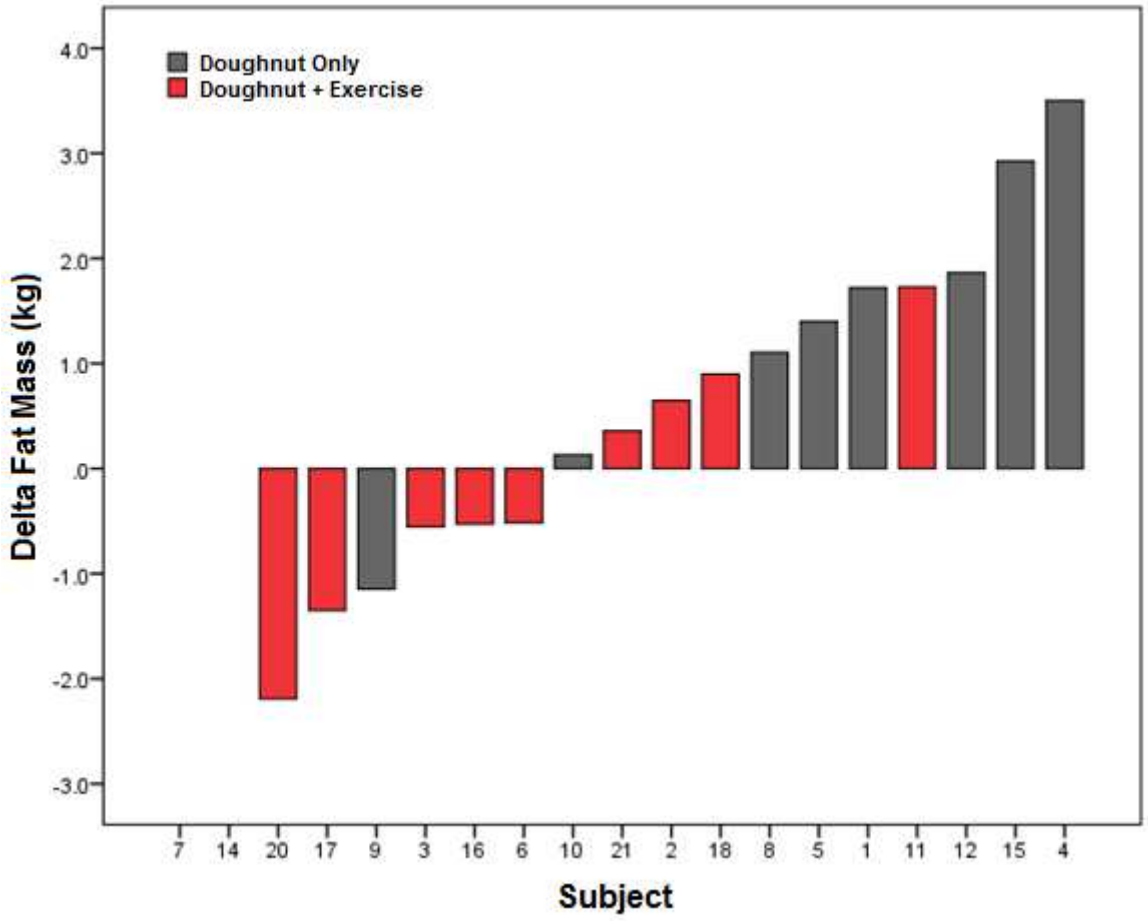

Figure 2. Individual change in fat mass by group over time. There was a significant difference in change in mean fat mass between the doughnut + exercise group (-0.2±1.2) and the doughnut only group (1.5±1.5) (p=0.013).

Table 4

Endothelial Function

	Doughnut + Exercise (n=8)				Doughnut Only (n=9)			
Measure	Pre	Post	Δ	*p* value time effect	Pre	Post	Δ	*p* value time effect
FMD (%)	5.23±3.75	3.78±1.06	-1.45±3.83		5.75±4.00	7.32±4.47	1.20±4.43	
Baseline Diameter (cm)	0.39±0.03	0.42±0.03	0.03±0.02	p=0.001	0.37±0.04	0.38±0.03	0.01±0.03	
Peak Diameter (cm)	0.41±0.03	0.43±0.03	0.02±0.02	p=0.04	0.39±0.05	0.41±0.05	0.01±0.02	

Results are reported as Mean ± Standard Deviation.

Table 5

Blood Markers of Cardiometabolic Risk

	Doughnut + Exercise (n=8)			Doughnut Only (n=9)			
Measure	Pre	Post	Δ	Pre	Post	Δ	*p* value of Interaction
hsCRP (mg/L)	0.69±0.85	0.66±.342	-0.03±0.62	0.89±1.39	1.75±2.57	0.77±2.56	
NO (umol/L)	7.1±8.7	11.4±17.2	4.3±8.7	6.3±3.4	1.8±0.5	-4.4±3.3	p=0.014
AOX (mM)	2.6±0.4	2.7±.4	0.1±0.4	2.1±0.3	2.2±0.2	0.1±0.3	
CHOL (mg/dL)	153±28	160±31	6.5±16	145±43	139±40	-4±11	
LDL-c (mg/dL)	92±28	95±30	3±12	82±35	78±36	-4±11	
HDL-c (mg/dL)	51±13	56±16	5±6	52±11	51±8	-1±7	
TRIG* (mg/dL)	92±56	76±32	-16±29	86±30	100±42	14±27	p=0.036
Glucose (mg/dL)	87±9	87±7	0±4	91±6	93±8	2±4	
Insulin (mg/dL)	11.1±3.7	9.5±3.4	-1.6±1.5	9.6±2.9	11.6±5.9	2.0±4.6	p=0.039
Insulin Sensitivity	2.1±1.0	1.8±0.8	-0.3±0.3	2.1±0.9	2.7±1.5	0.5±1.2	p=0.05

Results are reported as Mean ± Standard Deviation.
*(n=7) in the Exercise Group; omitting 2 statistical outliers

glucose, insulin, and insulin sensitivity are shown in Table 5. There was a significant difference in serum TG between the doughnut + exercise group (-16.0 ± 29.7 mg/dL) and the doughnut only group (15.1 ± 24.13 mg/dL) (p=0.036). There were significant group differences in change in fasting insulin between the doughnut + exercise (11.1±3.7 to 9.5±3.4 uU/ml) and doughnut group (9.6±2.9 to 11.6±5.9 uU/ml) (p=0.039). Similarly, there was a significant improvement in insulin sensitivity, as calculated using the HOMA equation, between the doughnut + exercise (2.1±1.0 to 1.8±0.8) and the doughnut only groups (2.1±0.9 to 2.7±1.5) (p=0.05) though there was no significant change in fasting glucose between groups. There were no significant differences in total cholesterol, LDL, HDL within subjects or between groups in this study.

Chapter 5

DISCUSSION

In this study, exercise training 4 days per week, including 2 days of high-intensity interval exercise and 2 days vigorous-intensity, steady-state exercise, were utilized to examine the efficacy of exercise to ameliorate the expected undesirable health effects of a fat-sugar supplemented diet in otherwise healthy, college-aged males. Following three weeks of sustained overfeeding of approximately 600 calories per day, 6 days per week, in the form of doughnuts, exercise prevented weight gain and an increase in body fat. The results on cardiovascular risk markers were mixed, largely due to the fact that the donut-supplemented diet did not negatively impact most blood markers examined in the control group.

It was hypothesized that the consumption of three dozen doughnuts over 3 weeks would adversely impact cardiometabolic risk markers, and that the regular exercise sessions would prevent this from occurring. Unexpectedly, the doughnut consumption had a relatively modest effect on most blood markers of cardiometabolic risk, with the major exception being the significant gain in body fat. On the other hand, exercise not only prevented deterioration in all risk markers studied, but rather resulted in significant improvements in several outcome measures, including insulin sensitivity, nitric oxide bioavailability, and serum triglycerides. These results are consistent with previous findings that suggested that the health benefits of exercise and/or aerobic fitness are independent of diet quality (Heroux et al, 2010; Huffman et al, 2012; Kouki et al, 2012), and also demonstrate that some cardiometabolic risk markers can be improved with

regular, vigorous exercise even when accompanied by the addition to the habitual, *ad libitum* diet of a fat-sugar supplement in the form of doughnuts.

Body Composition

Increases in body weight and body fat could be expected as a result of adding ~11,600 kcal, mostly from fat and sugar, to an *ad libitum* diet. After three weeks of adding the fat-sugar load to the diet, participants in the doughnut + exercise group experienced no weight gain, and no change in body fat. It is important to note that the estimated energy value of the exercise sessions (~4,360 kcal) was only about 38% of the energy value of the 3 dozen doughnuts (~11,600 kcal). Even if the estimated energy cost of the excess post-exercise consumption (EPOC) is included (~10% of the exercise energy expenditure, or ~436 kcal), the cumulative energy expenditure of the exercise training sessions corresponds to only about 41% of the energy value of the 3 dozen doughnuts. This suggests that exercise may have an "energy balance" effect beyond that attributable to just the energy value of the exercise itself. It is possible that weight maintenance during a period of overfeeding is attributable in part to the effect of exercise on increased sensitivity to satiety hormones (discussed below).

Similar results have been shown in rat studies examining the effects on overfeeding, which found that though increased energy expenditure through exercise training was compensated for by a significantly higher caloric intake in the training groups, trained animals consuming a high-fat diet gained significantly less weight than their sedentary control counterparts (Gollisch et al, 2009). In fact, a number of previously published reports support the effectiveness of exercise as a means to prevent weight gain during periods of overfeeding (Gomez-Merino, Drogou, Guezennec,

Chennaoui, 2007; Boutelle, Kirschenbaum, Baker, Mitchell, 1990; Baker & Kirschenbaum, 1998).

The doughnut only group gained ~1.4 kg body fat. Using the standard values for the energy equivalent of fat tissue (9540 kcal; King et al 2009), this corresponds to an increase in energy value of body fat of approximately 13,350 kcal. This is fairly close to the energy value of the 3 dozen doughnuts, but also suggests that consuming the doughnuts may have increased energy consumption slightly during the 3-week intervention. Consuming carbohydrates increases carbohydrate utilization and suppresses fat oxidations (Jecquier, 1993). Thus the consumption of ~11,600 kcal of mostly sugar and fat over three weeks, while maintaining an otherwise *ad libitum* diet, could be expected to increase fat storage. The ~1.4 kg fat gain experienced by the doughnut only group is consistent with this expectation.

Dietary intake during this study similarly parallels that of the frequent "overconsumptioin" that may occur during a holiday season. The time between Thanksgiving and New Year's is notorious for an increased propensity to over consume, especially high sugar/fat foods. For most adults, there is a slight increase in weight over time, with the average weight gain in young adults ranging from 0.2 to 0.8 kg per year. Of that weight gain, there was a significant increase (0.48±2.22 kg) during the holiday period but not during the pre or post-holiday periods (Yanovski, Sovik, Nguyen, O'Neil & Sebring, 2000). That is to say, weight gain that occurs during periods of "overeating," such as during the holiday season, apparently is not subsequently lost (Yanovski et al, 2000). This could explain in part the pattern of adult weight gain that occurs in the United States. The results of the present study suggest that exercise may be an important

behavior that could minimize, or prevent, unintended weight gain during short (~several weeks) periods of overconsumption relative to energy expenditure.

In the holiday weight gain study of Yanovski et al (2000) only reported changes in activity and hunger' were significantly associated with increased percentage of weight gain. Those who reported lower activity or greater levels of hunger gained the most weight over the holiday period. Even minimal weight gain experienced by normal weight subjects has a compounding effect if not lost from year to year. This trend has been shown in the National Health and Nutrition Examination Survey follow-up study that among adults 25 to 44 years old, body weight measured at 10-year intervals increased by an average of 3.4 percent in men and 5.2 percent in women (Williamson, Kahn, Remington, Anda, 1990; Williamson, 1993).

In studies with similar outcomes, exercise training prevented the effects of a high-fat diet on characteristics of adipose tissue, with a more pronounced impact on visceral than subcutaneous fat tolerance (Gollisch et al, 2009; Xu et al, 2011). It has been shown that one channel through which exercise acts is by increasing the brown adipocyte-specific gene (UCP1) expression as well as increasing mitochondrial density (Xu et al, 2011). This expression is a critical step in activating brown adipogenesis. Because brown adipose tissue acts similarly to skeletal muscle in the way of energy metabolism, an increase in brown adipose tissue could potential increase fat oxidation for the purpose of fuel availability. Although the role of exercise in brown adipose tissue development and metabolism in humans remains speculative, considerable variation in brown adipose tissue quantity and distribution has been shown to exist in young men (van Marken Lichtenbelt et al, 2009).

Exercise may be capable of triggering phenotypic changes in both brown and white adipose tissue that would cause it to be more metabolically active. The cellular pathway by which this change in brown adipose is theorize to occur is by the increase of the brown adipocyte-specific protein UCP1 expression. This enhances the oxidative phosphorylation through increased mitochondrial number and dissipation of energy as heat, improving the energy balance, and ameliorating adiposity and its complications (Xu et al, 2011). Exercise has also been shown to reduce inflammation in visceral adipose tissue and inflammatory cytokines such as TNF-α and MCP-1 in white adipose tissue despite consuming a high fat diet (Gomez-Merino et al, 2007; Bradley, Jeon, Liu & Maratos-Flier, 2008). In the current study, inflammatory cytokines were not measured; however, high fat/sugar diet and/or exercise training did ameliorate weight gain and prevent increases in adipose tissue. Though more research is needed to support the findings regarding the alteration of adipose tissue function through exercise, the potential health implications for improving the viability of brown adipose tissue is apparent because high levels of brown fat (activity) may provide protection from diet-induced obesity, diabetes, and insulin resistance (Hamann, Flier & Lowell, 1996).

Endothelial Function

The brachial artery flow-mediated dilation (FMD) results were unexpected. Exercise training has been reported to increase FMD, with an effect that is most apparent after 2 to 4 weeks of training (Tinken, 2008). FMD, reflecting functional changes in the endothelium, may decrease after this initial adaptation as a result of structural remodeling of the artery. In fact, FMD after 8 weeks was no different than baseline FMD in the study of Tinken et al (2008). This structural remodeling is

manifested by an enlargement of the arterial diameter. In the present study, the baseline arterial diameter increased significantly more after the 3-week intervention in the exercise trained group. Because FMD is inversely related to arterial diameter, the lack of change (actually, a trend for slight decrease) in FMD in the exercise-trained group may reflect some level of arterial remodeling in the exercise group. If so, the present results suggest that arterial remodeling may occur much more rapidly than previously thought.

The lack of change in FMD must also be interpreted from the perspective of the actual changes in arterial diameter during the procedure. FMD is calculated as the percent difference between the baseline diameter (prior to cuff inflation) and the peak diameter during the 5-minute measurement period after cuff deflation. The exercise group experienced a significant increase in baseline diameter (reflecting arterial remodeling), and also had a significant increase in peak diameter after cuff release (suggestive of augmented endothelial function). The FMD (i.e., percent increase in diameter) did not change (actually decrease slightly, but not statistically significantly) because the baseline diameter increased by more than the peak diameter. Thus the current results suggest that both structural and functional changes in the brachial artery may have occurred as a result of exercise training that opposed any potentially detrimental effects of the doughnut-supplemented diet.

The nitric oxide results support this possibility. Nitric oxide availability increased significantly in the exercise group, and nitric oxide plays a critical role in endothelial function. By contrast, nitric oxide availability decreased in the control group. This suggests that despite consuming a high fat/sugar load in the form of doughnuts for three weeks, four days per week of mixed-intensity exercise training was sufficient to mitigate

all negative dietary effects while triggering a concomitant increase in nitric oxide availability. Previous studies have shown similar findings (de Morales, Davel, Rossoni, Antunes & Zanesco, 2008; McCarthy, Farney, Canale, Dessoulavy, Bloomer, 2013). The results of the control group in the present study are consistent with studies indicating significant declines in nitric oxide availability in response to a moderately high-fat load (Huang et al, 2011; Tamura et al., 2009). A significant decline in nitric oxide bioavailability may result from a decrease in nitric oxide production or the scavenging of nitric oxide by reactive oxygen species (ROS). In one study the decrease in nitric oxide was coupled with an increase in protein nitration which could potentially have adverse effects on tissue function (Huang et al, 2011).

The significant changes in nitric oxide availability without a subsequent significant change in FMD calls attention to additional contributing mechanisms involved in vascular reactivity. Potential mechanisms include antioxidant concentration, essential fatty acids, and oxidative stress. The present study revealed no significant group differences in total antioxidant capacity across time. Antioxidant capacity has been reported to be increased within ~16 h of an acute exercise bout, with higher-intensity exercise being more effective than moderate-intensity exercise (Tyldum et al, 2009). In the present study, post-training fasting blood samples were drawn approximately 48 hours after the last exercise session. An increase in total antioxidant capacity of the blood resulting from an acute exercise bout may be only transient, with normalization occurring within 48 hours. If so, this could explain the current finding of no increase in total antioxidant capacity of the blood after training. Because total antioxidant capacity has also been reported to correlate strongly with FMD (Tyldum et al, 2009), a transient

effect of exercise on total antioxidant capacity may help explain the lack of a significant change in FMD in the group that received exercise training.

A lack of increase in antioxidant capacity in the exercise group could be related to the significant rise in Nitric Oxide. Nitric oxide, one of two primary free radicals generated during exercise, is highly reactive and easily converted to ROS. Unstable free radical species attack cellular components which cause damage to lipids, proteins, and DNA. These changes can trigger a number of disease pathologies. Contraction of skeletal muscle and increases in ROS generation has been shown to have dose response relationship, with greater production coming from higher intensities or long duration (Powers & Jackson, 2008). Because these ROS lead to muscle fatigue, the myocytes maintain at defense system integrating both exogenous and endogenous antioxidant molecules to mitigate pro-oxidative effects. This theory suggests that the increased nitric oxide production led to increased production of oxidants which led to increased utilization of available antioxidants.

Another potential confounder of the results is habitual diet. Although participants were asked to refrain from taking nutritional supplements including fish-oil during this study, dietary consumption of essential fatty acids was not measured. Previous research has shown that 1g eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was able to preserve endothelial function after a high-fat meal in a healthy population (Fahs et al, 2010). Other studies have also shown higher doses of omega-3 fatty acids to preserve both endothelial-independent (Armah et al. 2008) and endothelial-dependent function (Vogel et al. 2000; West et al., 2005) after a high fat meal. Although consumption of

individual dietary fatty acids was not measured in the current study, the habitual fat intake was similar between groups (~77 g/day; ~28% of total energy).

Cardiovascular Risk Factors

Overfeeding, either by carbohydrate, fat or a combination of both has been shown to adversely affect cardiovascular risk factors. In the present study, most blood variables did not change, and this may reflect the population studied—reasonably healthy young males with normal blood pressures and normal blood lipid and glycemic values. It is also possible that the fat-sugar supplement used in the current study--doughnuts--may not have incurred a large enough increase in overall saturated fat intake from baseline to bring about significant changes in either serum lipids or endothelial function. .

The consumption of three dozen doughnuts increased saturated fat intake by over 300g, or by 17g/day on the 6 days in which the donuts were consumed. Increased saturated fat intake has been shown to increase ectopic liver fat (Sevasianova et al, 2012). The ratio of saturated fatty acids to essential fatty acids in serum, and very low-density lipoprotein triglycerides also increased significantly. Similar to changes in fat and body weight, additional overfeeding studies have addressed the development of dyslipidemia which has been documented as a result of overconsumption of sugar, even when substituted calorie for calorie for fat (Parks & Hellerstein, 2000). An important mechanism is de novo lipogenesis, the synthesis of the saturated fatty acid palmitate, from glucose, fructose, or both. The upregulation of this process is key because triglyceride synthesis and secretion by the liver is increased by the generation of palmitate in conjunction with glycerol and malonyl coenzyme A (Hudgins, 2012). Some studies have seen up to a fivefold increase in liver triacylglycerol.

The triglyceride response in the control group in the present study is consistent with these previous findings. A fat-sugar supplement would be expected to suppress lipid utilization, which could result in both an increase in fat storage as well as an increase in triglyceride formation. The increase in triglycerides and gain in body fat in the doughnut only group can be seen as a natural consequence of an ad libitum diet supplemented with excess fat and sugar. The reduction in triglyceride concentration in the exercise group, despite the consumption of the ~11,600 kcal in the form of doughnuts, highlights the beneficial impact of exercise on regulation of body fat and reducing fasting triglycerides.

The significant decrease in triglycerides in the exercise group may be a function of increased skeletal muscle lipoprotein lipase protein expression which increases the hydrolysis of circulating triglycerides (Freese, Levine, Chapman, Hausman, Cureton, 2011; Seip, Angelopoulos & Semenkovich, 1997). Increased lipoprotein lipase activity, which aids in triglyceride clearance, peaks 4 to 8 hours after exercise and returning to baseline values within 24 hour post exercise. These findings are similar to those in previous studies; however, the short duration of this study may have been too acute in nature to detect significant changes seen by other studies (Freese et al, 2011; Perry, Heigenhauser, Bonen & Spriet, 2008). It is possible that the particular demographic had a sufficient level of metabolic flexibility to accommodate the short period of overfeeding experienced in this study.

Glucose and Insulin

In the present study, the exercise training resulted in a significant improvement in fasting insulin and insulin sensitivity (HOMA) despite consumption of a fat sugar supplemented diet. In contrast, the doughnut only group experienced an increase in both

measures. The improvement in insulin sensitivity was seen despite any significant changes in blood glucose levels. These findings are consistent with previous findings which have shown that insulin sensitivity can be improved independently from improvements in serum lipids (Bradley et al, 2008). In addition to aiding in the control of blood glucose, insulin is adipogenic. At the periphery, insulin is influential in increasing body fat mass and stimulating the production and secretion of leptin, a potent satiety hormone that acts centrally to reduce food intake and increase energy expenditure. Insulin has been identified as a regulator of leptin because the fluctuations in plasma insulin concentrations in response to feeding parallel those of plasma leptin concentrations.

Recent research has explored the impact of exercise and the feeding of various high-fat or high-sugar diets on satiety hormones such leptin, adiponectin and ghrelin. Leptin is an adipose-derived protein hormone that plays a critical role in the regulation of energy balance. The suppression by leptin has more of a chronic control over appetite, that if suppressed can potentially lead to increased susceptibility to overfeeding and, resultantly, weight gain (Kieffer & Habener, 2000). Circulating leptin levels are directly proportional to the percentage of body fat and fluctuate with changes in caloric intake and the amount of energy stored in adipocytes to maintain energy homeostasis (Chan et al, 2003). Leptin acts on the receptors in the hypothalamus to suppress appetite by binding to other chemical feeding stimulants such as neuropeptide Y.

Based on the insulin and leptin interaction, increased insulin concentrations in addition to increased adipose tissue should result in an increase in leptin and a subsequent decrease in appetite. Subjects in this study reported increased appetite at the onset of the

doughnut consumption. Similar to the adaptation of the body toward hyperinsulinemia, if a period of sustained high leptin levels occurs, a subsequent decrease in leptin sensitivity has been shown (Myers, Cowley & Münzberg, 2008). This leptin resistance could potentially lead to a blunted response from leptin in response to a high caloric feeding.

Similar to leptin, adiponectin is a protein hormone secreted from adipose tissue. This hormone aids in the control of energy metabolism through its critical role in carbohydrate regulation and lipid catabolism. More specifically, this hormone is responsible for decreasing gluconeogenesis, increasing glucose uptake, increasing β-oxidation, and facilitating triglyceride clearance (Díez & Iglesias, 2003; Nedvídková, Smitka, Kopský & Hainer, 2005; Vasseur, Leprêtre, Lacquemant & Froguel, 2003). In contrast to leptin, adiponectin levels are inversely correlated to body fat percentage with weight reduction significantly increasing circulating levels.

One explanation for the body weight responses of the exercise and control groups may be related to the location of lost or acquired body fat respectively. Previous research has indicated that only subcutaneous leptin mRNA was elevated in sedentary high-fat-fed rats, suggesting that subcutaneous adipose tissue may play a predominant role in diet-induced increases in serum leptin (Gollisch et al, 2009). The current study did not measure compartment changes in body composition by subcutaneous or visceral. Another feasible mechanism contributing to these research findings is an alteration in sensitivity within the central signaling pathways of insulin and leptin. Research has supported the hypothesis that hypothalamic effects of leptin might be mediated by phyosphatidylinositol 3-kinase (PI3K) signaling (Niswender et al, 2001). Like insulin, leptin activates PI3K in hypothalamic neurons and this activation is required to reduce

food intake (Zhao et al, 2002; Niswender et al, 2001). These findings support the theory that any interruption in the activation of insulin or leptin signaling pathways could attenuate the ability of adiposity-related hormones to regulate energy homeostasis. Human obesity seems to be characterized by resistance to adiposity signals within the hypothalamus, such as insulin and leptin, as suggested by markedly increased serum insulin and leptin levels, reflecting an increase in body adipose mass (Bagdade, 1967; Considine et al, 1996). Despite this increase in adiposity signals, food consumption remains normal or high, which is a comparable metabolic dysfunction observed in type 2 diabetes.

Acutely, impairment of insulin sensitivity has been shown to occur after only 3 days of inactivity and overfeeding preceding changes in body composition (Knudsen et al, 2012). After 7 days the metabolic consequences had worsened with an elevated fasting insulin level, a marked decrease in insulin sensitivity, and a compensatory increased insulin response to an oral glucose load to maintain glycemic control, signifying a loss of metabolic flexibility. In a rodent model, a high-fat diet increased average blood glucose levels in sedentary rats but not in exercise-trained rats on the same diet (Gollisch et al, 2009).

The aforementioned development of insulin resistance may partially be explained by increased liver fat, possibly in association with maximal lipid storage capacity in visceral adipose tissue. The increase in *de novo* lipogenesis was significantly correlated with that in liver fat content. These results provide support for the measurement of liver and plasma triglycerides and *de novo* lipogenesis after a high sugar load in populations at greatest risk for fatty liver. Consequently, there is increased rationale for making dietary

recommendations to restrict sugars while targeting those at greatest risk of the identified pathogenic mechanisms of action.

Fructose has also been recognized as being independently lipogenic because it is metabolized primarily in the liver (Bray, 2007). Studies have shown that diets with high fructose intake result in lower post-prandial circulation of insulin and leptin as well as elevated ghrelin (Teff et al, 2004). This hormone cocktail has been suggested to contribute to increased weight gain and the development of non-alcoholic fatty liver disease (Ouyang et al, 2008). More specifically data shows that a 2% increase in body weight and similar increases in subcutaneous and visceral adipose tissue were accompanied by a 27% increase in liver fat measured (Sevastianova et al., 2012). Other potential ramifications of this metabolic imbalance include increased oxidative stress, inflammation, and insulin resistance (Stanhope et al, 2009). The implication that an indefinite overconsumption of calories from dietary sugar can cause or exacerbate fatty liver provides further support to public health recommendations to limit discretionary calories from dietary sugar intake.

Conclusions

The results of this study support existing research demonstrating that exercise can significantly attenuate a number of negative metabolic and weight-related changes that are associated with overfeeding. The most compelling result of this study is that 4 exercise sessions per week, each requiring ~30 minutes, were sufficient prevent weight and body fat gain during a 3-week period of consuming ~11,600 kcal in the form of doughnuts, as a supplement to normal diet in young men. This prevention of weight and fat gain occurred despite the fact that the cumulative caloric cost of the exercise sessions

was only ~41% of the energy value of the 3 dozen doughnuts consumed. Furthermore, increases in insulin sensitivity and total nitric oxide availability were observed in the exercise group despite the addition of ~11,600 kcal, largely as fat and sugar, to the subjects' habitual diets. Although consuming 3 dozen doughnuts did not appear to have consistent adverse effects on a number of cardiometabolic risk markers in the control group that did not exercise, the significant gain in body fat, averaging 1.5 kg in just 3 weeks, suggests that regular exercise, especially during periods of increased energy consumption (e.g., holidays) may be essential for weight control and prevention and/or minimization of adult weight gain. Whether these results extend to women, and other populations that are at increased risk for obesity, diabetes and/or cardiovascular disease, remains to be established.

Strengths and Weaknesses

The supervised nature of the exercise training, in which all subjects in the exercise group completed 100% of their planned supervised exercise sessions, is a strength. The fact that the control group did not change VO_2peak is evidence that they did not engage in any unmeasured activity that might have affected their fitness levels. Also, the manner in which the doughnuts were disbursed is a strength, as this enhanced compliance. Lack of control over habitual diet is a weakness, but pre-intervention diet records indicated that both groups were similar in total energy intake and macronutrient composition of the diet. The results of the study may be specific to young, healthy males, with relatively normal values for cardiometabolic risk markers studied. Also, the exercise training sessions were vigorous in nature. Whether less intense exercise sessions could produce the same result remains to be established. Follow-up studies on women, and other at-risk

populations need to be performed. Furthermore, the study had a relatively small sample size, although it a priori adequately powered for the primary outcome (FMD), and most previously published studies of this nature have similar sample sizes.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

Arizona State University Office of Research Integrity and Assurance P.O. Box 871103 Tempe, AZ 85287-1103 Phone: 480-965-6788 Fax: 480- 965-7772		*For Office Use Only:* H.S. #: Pre-Reviewer:

BIOSCIENCE APPLICATION HUMAN SUBJECTS

PROTOCOL INFORMATION

1.
Protocol Title: Effects of a fat-sugar supplemented diet, with and without exercise training, on endothelial function, blood pressure, and blood markers of cardiovascular risk

Date of Request: September 26, 2012

PRINCIPAL INVESTIGATOR (PI)

Please note that the PI's CV and human subject's protection training certification must be attached with this application.

2.
Name and Degree(s): Glenn Gaesser, PhD.

Department/Center: Exercise and Wellness/Healthy Lifestyles Research Center

Mailing Address: 500 North 3rd Street, Phoenix, AZ 85004

Email: Glenn.gaesser@asu.edu Phone #: 480-727-1884 Fax: 602-496-1873

University Affiliation:
☒ Professor
☐ Associate Professor
☐ Assistant Professor
☐ Instructor
☐ Other: Please specify. ("Other" categories may require prior approval. Students cannot serve as the PI)

CO-INVESTIGATORS (CO-I)

3. Name	Study Role	Affiliation	Department	Email/Tel/Fax	Student (y/n)
Laurie Black	Research Assistant	ASU	PANW	laurie.e.black@asu.edu	Y
Brandon Sawyer	Research Assistant	ASU	PANW	bjsawyer@asu.edu	Y
Dharini Bhammar	Research Assistant	ASU	PANW	dbhammar@asu.edu	Y
Wesley Tucker	Research Assistant	ASU	PANW	wesley.tucker@asu.edu	Y

PROJECT FUNDING

4a) How is the research project funded? (A copy of the grant application **must** be provided prior to IRB approval)

☒ Research is **not funded** (Go to question 5)
☐ Funding decision is pending
☐ Research is **funded**

b) What is the source of funding or potential funding? (Check all that apply)

☐ Federal ☐ Private Foundation ☐ Department Funds
☐ Subcontract ☐ Fellowship ☐ Other

c) Please list the name(s) of the sponsor(s):

d) What is the grant number and title?

e) What is the ASU account number/project number?

f) Identify the institution(s) administering the grant(s):

SUMMARY OF PROTOCOL

Please include a summary for each of the questions. Use as much space as necessary **AND** attach a copy of the study proposal or protocol. If you attach a copy of the full proposal, place page and paragraph numbers from the proposal next to each question in this section to show precisely where information pertaining to each question can be found. Please note that information should be **consistent** between the proposal, consent form, and IRB application. **FOR ALL OF THE QUESTIONS, WRITE YOUR ANSWERS ON THE APPLICATION RATHER THAN SAYING SEE ATTACHED.**

5a) What is the hypothesis?

The objective of the study is to determine if exercise training can prevent the anticipated deleterious effects of a fat-sugar supplemented diet (in the form of two doughnuts per day, 6 days per week, for 3 weeks) on endothelial function and blood markers of cardiovascular risk. We hypothesize:

H1: Fat-sugar supplemented diet will impair endothelial function and result in a worsened cardiovascular risk profile as assessed by fasting blood lipid profile, insulin, glucose and antioxidant capacity.

H2: Exercise training will prevent deleterious effects of a fat-sugar supplemented diet.

b) Describe study procedures and methodologies.

Visit 1 (Screening):

All participants who respond to the recruitment flyer will be provided a copy of the consent form to read, and will be informed that any questions they have about the study will be answered by study personnel. All aspects of the study will be explained and written consent will be obtained. Participants will fill out a physical activity readiness questionnaire (PAR-Q) to ensure that they are suitable candidates for enrollment. If they answer "yes" to any of the questions, they will not be allowed to participate. If they answer "no" to each question, they will be allowed to participate. Those who decide to participate after signing this consent form we will undergo a flow-mediated dilation (FMD) procedure (see below) to make sure we can adequately obtain an ultrasound image of each participant's brachial artery. Participants will complete an automated self-administered 24-hour diet recall (ASA24; free online service provided by the National Institutes of Health (National Cancer Institute)).

Subsequent Visits:

Participants who agree to take part in the study will report to the Healthy Lifestyles Research Center in ISTB3 on the ASU Polytechnic Campus for all visits. Participants randomly assigned to the Control group will have 11 additional visits beyond the screening visit (visits 2 through 12) over a 3-week period. Participants randomly assigned to the exercise group will have a total of 14 visits beyond the screening visit (visits 2 thorough 15) over a 3-week period. Visits are described in detail below.

Visit 2 (Baseline testing; both Control Group and Exercise Training Group): The participants will report for baseline testing at the ISTB3 building following an overnight fast (nothing but water after 10 PM).

Participants will first have resting blood pressure measured, followed by a measurement of brachial artery flow-mediated dilation (described later). Following this measurement, participants will have a fasting blood draw.

Participants will have body composition measurements taken (BOD POD; described later). All participants will perform a VO_{2max} test on a treadmill to determine maximal oxygen uptake (described later).

Participants will be randomly assigned to either the exercise or control group.

Visits 3 through11 (Control Group)

These visits will be for picking up the doughnuts that will be provided to each participant during the 3-week study. Participants will pick up doughnuts at the ISTB3 laboratory on Monday, Wednesday and Friday of each week. Details are described below.

Visits 3 through14 (Exercise Training Group only):
The participants in the exercise training group will report to the ISTB3 building for exercise training. Participants will train four (4) days per week for three (3) consecutive weeks. Training sessions are not required to occur on consecutive days. All exercise training will be performed on a motor-driven treadmill or a cycle ergometer. The aerobic exercise training protocol will consist 2 sessions/week of continuous exercise and 2 sessions/week of interval exercise performed on either a cycle ergometer or treadmill. Intensity on the treadmill will be achieved by walking on an incline or running or cycling against weighted resistance on the cycle ergometer. The exercise protocols are as follows:

- 2 days per week of continuous exercise: 30 minutes at a heart rate associated with 75% $V0_2$max determined from the baseline cycle test.
- 2 days per week of high-intensity interval exercise:
- Ten 1-minute intervals at 90-95% of maximum heart rate, separated by 1 minute of active recovery at 60% of maximum heart rate (Little et al, 2011).
- Four 4-minute intervals at 90-95% of maximum heart rate, separated by 3 minutes of active recovery at 60% of maximum heart rate (Wisloff et al, 2009).

Each exercise session will being with a 5 minute warm-up (50-60% of maximum heart rate), and finish with a 5-minute cool-down (50-60% of maximum heart rate).

Final Visit (Visit 12 for Control Group; Visit 15 for Exercise Training Group):
Approximately 48 hours following the last training session, the participant will report to the ISTB3 building. The testing procedures at this visit will be identical to those at Visit 2.

The procedures for testing are described in detail below:

- Food Frequency Questionnaire:

The ASA24 allows study participants to report their intake for the previous day from midnight to midnight. The Respondent application guides the participant through the completion of a 24HR for the previous day from midnight to midnight using a dynamic user interface.

- Brachial Artery Flow Mediated Dilation (BAFMD):

This is a non-invasive assessment of the ability of the brachial (upper arm) artery to dilate in response to an increase in blood flow, and is assessed using ultrasound. This procedure is performed while the subject is lying supine on a padded ultrasound table. All measurements are made on the non-dominant arm. A blood pressure cuff is positioned on the subject's forearm. After recording baseline ultrasound measures on the upper arm, the blood pressure cuff is inflated to 240 mmHg for 5 minutes. The cuff is then deflated rapidly and brachial artery blood flow and arterial diameter are measured continuously for 5 minutes using the ultrasound probe. These procedures conform to the published guidelines of the International Brachial Artery Reactivity Task Force (Corretti

MC, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *Journal of the American College of Cardiology*, 2002; 39: 257-265).

- Blood Draw:

Subjects will be asked to report for each blood draw (visits 2 and 12 for Control group; visits 2 and 15 for Exercise Training group) in the morning following an overnight fast (i.e., nothing but water after 10 PM). Intravenous blood will be draw via one of the common antecubital fossa veins (cephalic, median cubital, or basilic vein) for measurement of blood lipids, glucose, insulin, and antioxidant capacity. All blood draws will be performed by a certified phlebotomist. Approximately 15ml (3tsp) of blood will be collected for each blood draw.

- Resting blood pressure will be measured using an automated blood pressure machine while the participant is sitting quietly in a comfortable chair.

- Anthropometrics:

Subjects' weight will be measured on a standard Beam scale. Height will be assessed on a stadiometer. Body composition will be measured using a Bod Pod (air displacement plethysmography).

- VO_{2max} test:

Subjects will be equipped with a mask attached to a hose, and Polar heart rate monitor for the metabolic measurement device (Parvo Truemax 2400TM) to measure ventilation and respiratory gas exchange data and heart rate continuously. After collecting resting data for 2 minutes, subjects will pedal on a stationary cycle ergometer at a cadence of their choice at 50 watts for 5 minutes for the warm-up phase. After the warm-up phase, load will increase continuously by 30 watts/min every minute until the subject cannot continue. Verbal encouragement will be given to all subjects throughout the entire test. The highest oxygen uptake during the test will be taken as the peak VO_2.

- Doughnut consumption:

Each participant will be asked to consume 2 doughnuts per day, 6 days per week, for 3 weeks. Doughnuts will be purchased by the study researchers at a local Dunkin Donuts on Monday, Wednesday and Friday morning of each week. Participants will be required to come to the ISTB3 laboratory on Monday, Wednesday, and Friday of each week for doughnut pickup. This can be part of the regular exercise training visits for those in the Exercise Training group. On each occasion participants will be given 4 doughnuts to consume over the next two days. This will optimize compliance while ensuring that the doughnuts are fresh. Participants will be provided a menu of doughnut options so that each participant will be able to choose the types of doughnuts that he would like most to consume. All doughnuts will contain between 260 and 500 calories, of which at least 70% of total calories are fat and sugar.

- Dietary requirements:

Each participant will be encouraged to maintain his normal diet during the three weeks, other than consuming the 2 doughnuts per day provided by study researchers as a requirement for participating in the study.

c) What is the participant selection? Adult Males (ages 18-30 years)

d) What is the statistical design? Randomized control-group pretest-posttest design

e) Describe whether the study involves randomization to control/intervention groups. The study will have participants randomized into either the aerobic training or control group.

f) How will study results be used? Study results will be submitted in manuscript form to a peer reviewed journal.

DATA SAFETY MONITORING BOARD (DSMB)

6a) Does the study have a Data Safety Monitoring Board?
☐ Yes (prompt submission of DSMB reports is required) ☒ No

b) If no, what is the structure/plan to report serious adverse events to the ASU IRB?

All Adverse events will be reported to the IRB that could possibly (even remotely) be related to the study intervention. If the event is immediately life threatening, or potentially severely debilitating, then it will be reported to the IRB within 48 hours.

STUDY DURATION

7a) What is the expected duration of the study through data analysis? (Attach a timeline, if applicable) 1 year

b) What is the expected date that recruitment will begin? (must be after the submission date) October 1, 2012

STUDY SITES

8a) Where will the study be conducted? (Check all that apply)
☒ On campus (Please indicate building(s) and room number (s) when known) ISTB3, Rm. 183

☐ Off campus (Please provide location and letter of permission, where applicable)

b) Is this study being reviewed by another IRB? ☐ Yes ☒ No

Status of other IRB review: ☐ Approved ☐ Pending ☐ Not yet submitted ☐ Attached

INTERNATIONAL RESEARCH

9a) Does this study include an international site? ☐ Yes (list country) ☒ No

9b) If this is an international study, please provide a statement including the following items:

- The investigator's familiarity with the culture in which the study in taking place.
- Cultural norms and how this study may affect an individual's standing in his/her community.
- The standard of care in the community, how it differs from the proposed research procedures, and a plan for the continuation of care once the research is complete.

RESEARCH PARTICIPANT INFORMATION

10a) What are the inclusion criteria? (Use an expended list and attach a secondary sheet with explanation, where applicable. If you attach a secondary sheet, reference on which page the information can be found.)

Participants must be nonsmoking males between the ages of 18-30yrs and be physically able to participate in vigorous physical activity. The must also be willing to consume 2 doughnuts per day, 6 days per week, for three consecutive weeks. They must answer "no" to all questions of the Physical Activity Readiness Questionnaire.

b) What are the exclusion criteria?

Exclusion criteria:
- Does not meet age requirement
- Smoker
- Answers "yes" to one or more questions on the Physical Activity Readiness Questionnaire
- Unwillingness to sign informed consent
- Currently engaged in a regular exercise program
- Unwillingness to consume 2 doughnuts per day, 6 days per week, for 3 consecutive weeks
- Currently consumes greater than 4 donuts per week

c) Please explain recruitment procedures in detail. (A copy of the recruitment materials must be attached.)

Participants will be recruited through fliers placed on ASU campus. Electronic media will also be used by posting the research study flier on websites with volunteer opportunity postings.

d) What is the expected duration of participation of each participant? (total and at each session) 4 weeks total. The screening visit will take about 1 hour. The baseline (visit 2) and post-testing (either visit 12 or 15) sessions will require about 1.5 hours each. The doughnut pickup visits will last about 5 minutes each. The exercise training visits will last about 45 minutes each. Total time required for Control participants is approximately 4 hours 45 minutes. Total time required for Exercise Training participants is approximately 13 hours.

e) What is the expected number of individuals to be screened for enrollment? 40 people

f) What is the **maximum** number of individuals to be enrolled? (This includes individuals who drop out) 24

g) What is the approximate number of: 24 Males 0 Females

h) Indicate the age range of the participants that you plan to enroll in your study.
18 to 30

i) What is the race of participants? All race/ethnicities

j) Does the study target any of the following participants? ☐ Yes (please check all that apply) ☒ No

☐ Children (under 18)
☐ Decisionally impaired
☐ Prisoners or detainees
☐ Pregnant women
☐ Economically disadvantaged
☐ Persons at high risk of becoming detained or imprisoned
☐ Fetuses
☐ Patients, if yes – what is the status of their health? Type II diabetes
☐ Native Americans
☐ Non-English speakers (Include copy of all materials in language of participants)

k) If **any** of the above categories have been checked, please state how you will protect the rights and privacy of these individuals.

l) Does the study involve participants who have **low-literacy**? ☐ Yes ☒ No (If yes, please describe how investigators will ensure the participants' understanding of the research):

m) Does the study involve participants who are **students or faculty** of ASU? ☒ Yes ☐ No (If yes, please state the investigator's involvement in the participant's education/employment):

ASU faculty or students that meet the inclusion/exclusion criteria will be eligible to participate in the study. However, they will not be specifically targeted for recruitment. If ASU students, staff or faculty volunteer to participate, there will not be any effect on their employment or grade if they choose to withdraw from the study at time.

COMPENSATION

11. Will any type of compensation be used? (e.g. money, gift, raffle, extra credit, etc)
a) ☒Yes (Please describe what the compensation is): cash or gift certificate ☐ No (go to question 12)
Subjects assigned to the Control Group will receive $50 for completing both pre- and post-testing visits.
Subjects assigned to the Exercise Training group will receive $100 for completing all exercise training sessions and both pre- and post-testing visits.
b) Explain why the compensation is reasonable in relation to the experiences of and burden on participants.

Financial compensation is commensurate with time commitment. The higher compensation for those in the exercise training group is justified due to greater time commitment. All participants will receive 3 dozen doughnuts over the course of the 3-week study.

c) Is compensation for participation in a study or completion of the study? (Note: participants must be free to quit at any time without penalty).
☐ Participation ☒ Completion

d) If some or all participants are economically disadvantaged, explain how the compensation is provided in such as a way that participants cannot refuse the request to participate? This study will not be collecting information on socioeconomic status; all monetary compensation will be equal for all that complete the study.

RISKS AND BENEFITS

Please reference the proposal, where applicable and answer the questions below.

12a) What are potential risks to participants?

There is some risk associated with exercise, such as muscular soreness, musculoskeletal injury, tenderness and bruising from blood draws, and unexpected cardiovascular reactions to exercise, including shortness of breath, lightheadedness, fainting, chest pain, or death. Trained and certified personnel will oversee the exercises and watch for signs of distress and stop the exercises if necessary. There is a possible chance of weight gain as a result of daily caloric intake. Additionally, a fat-sugar supplemented diet may lead to impairment of arterial function and blood pressure.

b) What steps will the investigators take to reduce risks?

To prevent infection, standard procedures for collecting blood samples will be used as well as properly cleaning the area and using a tourniquet to enlarge the vein prior to the draw. Aerobic exercise protocol intensity will be relative to the participant's fitness level and physiological signs will be monitored to prevent or catch any abnormal responses to exercise. If necessary, first aid will be administered and 911 will be called if the incident requires such measures. All instruction based on American College of Sports Medicine guidelines.

c) What are any potential benefits to participants?

Free exercise testing, results of blood analyses, as well as brachial artery flow-mediated dilation (assessment of vascular health), free body fat testing.

d) Please note how the results of the study will affect the health and welfare of the general public.

The results of this study will be used to evaluate the potential for high-intensity aerobic exercise to mitigate the anticipated negative health effects of regularly consuming foods (e.g., doughnuts) with a high content of fat and sugar.

e) What are the types of incentives, if any, will participants receive?

Participants will be compensated for their participation

f) What are the costs, if any, to participants? (This should be mentioned in the consent form): No cost to participant

CONFIDENTIALITY

13a) Describe the steps you will take to ensure the confidentiality of the participants and data.

All information obtained in this study is strictly confidential unless disclosure is required by law. Data will be secured in a locked file cabinet in the faculty office of Glenn Gaesser. The results of this research study may be used in reports, presentations, and publications, but the name or identity of the subject will not be revealed.

b) How will you safeguard data that includes identifying or potentially identifying information (e.g. coding)?

In order to maintain confidentiality of records, we will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

c) When will identifiers be separated or removed from the data? At enrollment

d) Where on campus will you store the data and media and ensure its security (videotapes and/or audiotapes)?

Data will be coded and kept in a locked file cabinet or password-protected computer.

e) How long do you plan to retain the data? Indefinitely

f) How will you dispose of the data?

Hard copies of blood work and patient information will be shredded and disposed. Electronic copies of the data will be permanently deleted.

g) Is a certificate of confidentiality required? ☐ Yes ☒ No

HIPAA

14a) Are any of the data coming from covered entities under Health Insurance Portability and Accountability Act (HIPAA)? ☐ Yes ☒ No (If yes, please describe):

b) Is a data use agreement required? ☐ Yes ☒ No

c) Is a HIPAA Waiver of Authorization being requested? ☐ Yes ☒ No

DATA SOURCES AND USES

15a) Please check all the ways that you will obtain data: (Copies of written and oral questions must be provided for ASU IRB review and approval prior to implementation.)

- ☐ Interviews
- ☐ Focus Groups
- ☐ Medical Records
- ☐ Registries
- ☒ Questionnaires/Surveys
- ☐ Public Records
- ☒ Biological Specimens
- ☒ Other Experimental data collection in the laboratory

b) How will the data be used? (Check all that apply)

- ☒ Dissertation
- ☐ Thesis
- ☒ Results released to participants/parents
- ☐ Results released to agency or organization
- ☐ Other *(please describe):*
- ☒ Publication/journal article
- ☐ Undergraduate honors project
- ☐ Results released to employer or school
- ☒ Conferences/presentations

INFORMED CONSENT

16. Describe the procedures you will use to **obtain and document informed consent and assent**. **Attach copies of the forms that you will use.** In the case of secondary data, please attach original informed consent or describe below why it has not been included. Fully justify any request for a waiver of written consent or parental consent for minors. (The ASU IRB website has additional information and sample consent and assent forms.)

All individuals who inquire about participation in the study will be provided with a brief verbal overview of the study details, and will be apprised of the inclusion and exclusion criteria. Individuals expressing an interest to enroll will be provided with a copy of the consent form to read at their convenience. Those individuals wishing to continue with the enrollment will meet with one of the study investigators in order to complete the written consent process. A copy of the consent form is attached.

INVESTIGATIONAL NEW DRUG OR DEVICE

17a) Does this study involve an investigational new drug (within the meaning of 21 U.S.C. 355(i) or 357(d)) or a significant risk device (as defined in 21 CFR 812.3(m)?

☐ Yes ☒ No (If no, go to question 18. If unsure, go to: www.fda.gov/oc/ohrt/irbs/).

b) What is the drug or device?

c) Has the 30-day interval required for investigational new drugs and for significant risk devices elapsed, or has the FDA has waived that requirement?

d) If the 30-day interval has expired, has the FDA requested that the drug or device be withheld or restricted for use in human subjects? ☐ Yes ☐ No

DRUGS

18. What are the drugs to be used and in what dosage? (If no drugs will be used, please write N/A.) N/A

RADIATION

19a) Will ionizing radiation (x-rays and or radiopharmaceuticals) be used? ☐ Yes ☒ No (If yes, include a copy of the radiation certification).

b) Will non-ionizing radiation (MRI, ultrasound, lasers, ultraviolet) be used? ☒ Yes Ultrasound ☐ No

The ultrasound procedure will be performed by co-investigator Brandon Sawyer. He has performed more than 100 ultrasound procedures, which is the number recommended by the International Brachial Artery Reactivity Task Force (Corretti MC, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *Journal of the American College of Cardiology*, 2002; 39: 257-265).

BIOLOGICAL MATERIALS

20a) Will biological materials be collected from subjects or given to subjects? ☒ Yes ☐ No (If no, please skip to question 21)

b) Provide a description of the material (blood, tissue, vectors, antibodies, etc.) that will be used:

Fasting Lipid Panel, Blood glucose

c) If the study involves human blood, do you have the required ASU Biosafety disclosure on file? ☒ Yes ☐ No
(If yes, what is the biosafety disclosure number?) 09-204
(If yes, when and how are the samples to be destroyed? Note: an active protocol is required the entire period that the samples are retained)

The samples will be destroyed 6 months after the data analysis by putting the vials in a sealed plastic-lined biohazard waste box appropriate for blood and glass. The biohazard box will be labeled with contents, date, and originating location information.

d) Will any of the material being used in the study come from a third party? ☐ Yes ☒ No (If yes, attach copy of the Material Transfer Agreement if required.)

e) Does this study involve transfer of genetic material of animal tissue into humans? ☐ Yes ☒ No
(If yes, please cite the ASU Institutional Biosafety Disclosure number).

GENETIC ANALYSIS

21a) Does this study involve genetic analysis? ☐ Yes ☒ No (If no, please skip to question 22).

b) What sources of genetic material will be studied (blood, tissue, DNA)?

c) Please specify whether the genetic analysis involves pedigree, positional cloning, mutational polymorphism, or gene therapy research.

d) Please specify whether:
☐ Stored samples already exist
☐ Samples will be collected specifically for this study
☐ Stored samples already exist from a previously approved study
☐ Samples collected are part of a routine clinical procedure
☐ Samples are discarded, already existing, and de-identified

e) If stored samples will be used, did participants consent to the use of their stored sample(s)?
☐ Yes ☐ No (If yes, was the consent prospective to collection of the sample, or retrospective of the collection of the sample?)

f) Will any identifiers be maintained? ☐ Yes ☐ No (If yes, please specify)

g) When will samples be destroyed or discarded?

h) Are any of the diseases being studied considered preventable? ☐ Yes ☐ No

i) Is there a possibility of an incidental finding of a genetic condition? ☐ Yes ☐ No
If so, is there a plan to disclose this to the individual?

j) Will this information be kept confidential from third parties such as employers, or insurance companies, or will findings be included in the participant's medical record for clinical treatment?

k) How will other risks (e.g. discovery of information regarding paternity or ancestry) be disclosed to subject?

CONFLICT OF INTEREST AND COMMERCIALIZATION

22a) Does any member of the research team have a potential conflict of interest with this study that could affect study participants and/or study outcome? For more information about examples of conflicts of interests, please visit the ASU objectivity website: http://researchintegrity.asu.edu/coi
☐ Yes ☒ No (If yes, please describe and disclose in the consent form)

b) Does the PI or Co-I have a current conflict disclosure questionnaire on file at the ASU Office of Research Integrity and
Assurance?
☒ Yes ☐ No (Review ASU's objectivity in research policy: http://www.asu.edu/aad/manuals/rsp/rsp206.html)

c) Among the research team, is there any financial association that could affect any of the following: the study outcome, data analysis, enrollment of subjects, study design?
☐ Yes ☒ No (If yes, please describe and disclose in the consent form)

d) Are there any plans for commercial development related to the findings of this study?
☐ Yes (If yes, please describe.) ☒ No

e) Will the investigator or member of the investigator's family financially benefit if the findings are commercialized?
☐ Yes (If yes, please describe.) ☒ No

f) Will participants financially benefit if the findings are commercialized?
☐ Yes (If yes, please describe.) ☒ No

g) If there are conflicts of interests, please describe the ways in which the researchers will minimize harm to research subjects and/or the objectivity of research. N/A

TRAINING

23. The research team must document completion of human subjects training.

Please provide the date that the PI and Co-Investigators completed the training.

Glenn Gaesser	7-23-2012
Laurie Black	9-26-2012
Brandon Sawyer	6-13-2011
Dharini Bhammar	9-09-2011
Wesley Tucker	9-01-2010

(Attach a copy of the NIH Certificate for Human Participants Protections Education for Research Teams or CITI Training: http://researchintegrity.asu.edu/training/humans for the PI and Co-Investigators. Training must be within the past 3 years)

REQUIRED SIGNATURES

24. By signing this application form:
- I agree to accept responsibility for the rights and welfare of the human subjects involved with this study.
- I believe that the benefits outweigh the risks to the participants in this study.
- I agree to comply with Arizona State University IRB policies and procedures.
- I certify that, to the best of my knowledge, I am in compliance with the Department of Health and Human Services policies and procedures regarding the protection of human subjects.

Glenn Gaesser — 9/26/2012
Principal Investigator — Date

Attach a copy of the PI's CV unless one is already on file with the Research Compliance Office.

9/26/2012	Linda Vaughan
Department Chair/Dean	Date	Print Department Chair/Dean Name

(If the PI is the Department Chair or Dean, the application must be signed by another authorized Department/ School/College level Administrator)

FOR OFFICE USE:	This application has been reviewed by the Arizona State University IRB: ☐ Full Board Review ☐ Expedite Categories:_____ ☐ Exempt Categories: _____ ☐ Approved ☐ Deferred ☐ Disapproved ☐ Project requires review more often than annual Every months
Signature of IRB Chair/ IRB Member:	Date:

APPENDIX B

IRB MODIFICATION 1

Arizona State University
Office of Research Integrity
and Assurance
660 S. Mill Avenue Suite 315
Tempe AZ 85287-6111
Mail Code 6111
Phone: 480-965-6788
Fax: (480) 965-7772

For Office Use Only:
Date Received:

Modification Form Institutional Review Board (IRB)

INVESTIGATOR INFORMATION	
PROTOCOL TITLE: Effects of a fat-sugar supplemented diet, with and without exercise training, on endothelial function, blood pressure, and blood markers of cardiovascular risk	**HS # 1209008325**
PRINCIPAL INVESTIGATOR: Glenn Gaesser	**DEPARTMENT/ CENTER:** Exercise and Wellness/Healthy Lifestyles Research Center
CAMPUS ADDRESS: 500 North 3rd Street, Phoenix, AZ 85004	**PHONE:** 480-727-1884 **EMAIL:** **glenn.gaesser@asu.edu**
CO-INVESTIGATORS: Laurie Black, Brandon Sawyer,	
FUNDING STATUS: If project is funded or funding is being sought, provide list of all sponsors and grant numbers:	

TYPE OF MODIFICATION (CHECK ALL THAT APPLY) **Please attach any revised documents (forms, scripts, etc). Attach a brief summary of the proposed changes as well as a justification.**		
☒	New Procedures	Attach a description of the new procedures and a revised consent form. -VO2max test will be conducted on a cycle ergometer rather than a treadmill. -Training sessions will occur on a treadmill or a cycle ergometer (intensity remains the same as previously stated)
☐	Study Title Change	What is the new title?
☐	Change in Study Personnel	☐ Add (include the name, role, and contact information. Include copies of training certificates: http://researchintegrity.asu.edu/training/humans ☐ Delete

☐	Change of Site	☐ Add (include the name and location. If this changes the enrollment, that should be noted below.) ☐ Modify ☐ Delete
☒	Change in Enrollment	Attach a narrative justifying the change. If this will affect the consent, send a revised consent form as well. Participants will not be allowed to participate in this study if they currently consume greater than 4 doughnuts per week.
☐	Consent Change	Attach a copy and describe the change(s).
☐	Advertisement	Attach copies of the advertisement or announcement.
☐	Instruments (surveys, questionnaires, interviews, etc)	Attach copies of the proposed instruments and describe any changes from the approved protocol. If you are adding or deleting any instruments or items to an instrument, describe what the changes are and submit the revised materials.
☐	Other	Describe the changes. If this affects the consent process, submit a revised consent form.

SIGNATURE	
PRINCIPAL INVESTIGATOR:	Name (first, middle, last): Signature: Date:

APPENDIX C

IRB MODIFICATION 2

INVESTIGATOR INFORMATION	
PROTOCOL TITLE: Effects of a fat-sugar supplemented diet, with and without exercise training, on endothelial function, blood pressure, and blood markers of cardiovascular risk	**HS #**
PRINCIPAL INVESTIGATOR: Glenn Gaesser	**DEPARTMENT/ CENTER:** Exercise and Wellness/Healthy Lifestyles Research Center
CAMPUS ADDRESS: 500 North 3rd Street, Phoenix, AZ 85004	**PHONE:** 480-727-1884 **EMAIL:** **glenn.gaesser@asu.edu**
CO-INVESTIGATORS: Laurie Black, Brandon Sawyer, Wesley Tucker, Dharini Bhammer	
FUNDING STATUS: If project is funded or funding is being sought, provide list of all sponsors and grant numbers:	

TYPE OF MODIFICATION (CHECK ALL THAT APPLY) **Please attach any revised documents (forms, scripts, etc). Attach a brief summary of the proposed changes as well as a justification.**		
☒	New Procedures	Attach a description of the new procedures and a revised consent form. -Subjects will complete a Food Recall at the beginning and end of the study.
☐	Study Title Change	What is the new title?
☐	Change in Study Personnel	☐ Add (include the name, role, and contact information. Include copies of training certificates: http://researchintegrity.asu.edu/training/humans ☐ Delete

☐	Change of Site	☐ Add (include the name and location. If this changes the enrollment, that should be noted below.) ☐ Modify ☐ Delete
☐	Change in Enrollment	Attach a narrative justifying the change. If this will affect the consent, send a revised consent form as well.
☐	Consent Change	Attach a copy and describe the change(s).
☐	Advertisement	Attach copies of the advertisement or announcement.
☒	Instruments (surveys, questionnaires, interviews, etc)	Attach copies of the proposed instruments and describe any changes from the approved protocol. If you are adding or deleting any instruments or items to an instrument, describe what the changes are and submit the revised materials. Online version of ASA24 for research purposes
☐	Other	Describe the changes. If this affects the consent process, submit a revised consent form.

SIGNATURE	
PRINCIPAL INVESTIGATOR:	Name (first, middle, last):
	Signature: Date:

APPENDIX D

SUBJECT RECRUITMENT FLIER

ARIZONA STATE UNIVERSITY

Do You Like Doughnuts?

Healthy men (18 – 30 years old) are needed for a study looking at the health effects of consuming 2 doughnuts daily, 6 days per week for 3 weeks, with or without exercise training

Compensation
$50 - $100 plus 3 dozen doughnuts

This study takes place at the Healthy Lifestyles Research Center at the ASU Polytechnic Campus in East Mesa, and requires approximately 5 hours (control group) or 13 hours (exercise training group) of total time commitment over 3 weeks. **Your participation throughout the study is completely voluntary.**

Eligible participants must be **generally sedentary, nonsmokers**. Must be in generally good health, have no restrictions for participating in vigorous intensity physical activity, and must not be taking any medications for blood pressure, cholesterol, diabetes or a heart condition.

Please contact:
Laurie Black (614-359-0207; laurie.e.black@asu.edu)

APPENDIX E

CONSENT FORM

Informed Consent

Effects of a fat-sugar supplemented diet, with and without exercise training, on endothelial function and blood markers of cardiovascular risk.

INTRODUCTION

The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

RESEARCHERS

Glenn Gaesser, PhD, a professor, and Laurie Black, Brandon Sawyer, Dharini Bhammar and Wesley Tucker, doctoral students, in the Physical Activity, Nutrition and Wellness Program in the School of Nutrition and Health Promotion have requested your participation in a research study.

STUDY PURPOSE

Our primary objective is to determine whether exercise training can prevent the anticipated deleterious effects of a fat-sugar supplemented diet (in the form of two doughnuts per day, 6 days per week, for 3 weeks) on peripheral artery function, blood pressure, and blood markers of cardiovascular risk in healthy young men.

DESCRIPTION OF RESEARCH STUDY

If you decide to participate, then as a study participant you will join a study involving research on the effects of vigorous exercise training on blood lipids, blood glucose, and cardiovascular disease risk factors.

You are being asked to participate in this study because you are a relatively sedentary male and are, 18 - 30 years of age, in good health, and capable of performing vigorous physical activity. You must also be willing to consume two doughnuts per day, six days per week, for three weeks.

As a study participant you will have between 11 and 14 total visits to the Health Lifestyles Laboratory in ISTB3 room 181 on the Polytechnic campus of ASU. The number of visits depends on whether you are (randomly) assigned to the Control Group (11 visits) or Exercise Training Group (14 visits).

Visit 1 (screening):

Your first visit will involve coming to the test site on the ASU Polytechnic campus and filling out a physical activity readiness questionnaire that consists of 7 questions designed to assess whether participation in this study is appropriate for you. All aspects of the study will be explained to you, and we will answer any questions you may have. If you decide to participate after signing this consent form we will conduct the flow-mediated dilation (FMD) procedure (see below) to make sure we can adequately image your

brachial artery. During this visit you will also complete an automated self-administered 24-hour diet recall. This visit will take approximately 90 minutes.

Testing:
All testing will be completed at baseline and after 3-weeks for both the exercise training and control groups.

Baseline and 3-Week Testing (Visits 2 and 12 (Control Group); Visits 2 and 15 (Exercise Training Group):

- You will need to arrive at the laboratory in a fasted state (nothing but water after 10 PM)

- Height and weight will be measured using a standard scale.

- BOD POD:
  Your body composition (relative amounts of fat and lean tissue) will be determined by using a BOD POD, which is an air-displacement plethysmography procedure. This requires that you sit down in a fiberglass shell chamber (that looks something like a giant egg shell), and rest quietly for a few moments. You will wear a special bathing suit during this procedure. A clean bathing suit will be provided by researchers at ASU.

- Resting blood pressure will be measured using an automated blood pressure machine.

- Brachial Artery Flow-Mediated Dilation:
  This procedure involves taking ultrasound images of an artery in your upper arm before, during, and after a blood pressure cuff is inflated around your forearm. All measurements are made on your non-dominant arm. After lying quietly on a padded ultrasound table for 20 minutes, a blood pressure cuff will be positioned on your forearm. After recording baseline ultrasound measures on your upper arm, the blood pressure cuff will be inflated to a pressure of 240 mmHg (enough to stop blood flow to your wrist and hand), and kept in place for 5 minutes. You may experience a tingling feeling in your hand, which is normal. The blood pressure cuff will then be deflated rapidly and ultrasound measures will be taken for 5 minutes.

- A small amount of blood (approximately 3 teaspoons) will be drawn from one of the veins in your forearm for measurement of blood lipids, glucose, insulin, insulin sensitivity, and antioxidant capacity.

- Maximal Exercise Test: You will undergo a maximal exercise test on a bicycle. The test begins with you pedaling at a comfortable pace with little resistance for 5 minutes, after which the resistance will gradually increase until you reach a point

that you no longer can or want to continue. You will be asked to give an effort that is as close to maximal as you feel you are capable of. During this test you will wear a lightweight device that allows us to measure your breathing. This device consists of a shoulder/chest harness, which holds the gas analyzers in small pouches secured to the harness, and a facemask assembly that allows us to measure your breathing. The facemask is made of a flexible, rubbery-type material that fits over your mouth and nose and allows you to breathe naturally. The facemask is held in place with an elasticized net that fits over the back of your head and attaches to the facemask via elastic straps. You will also wear a Polar heart rate monitor, which is a lightweight, elasticized strap that you wear around your chest. <u>This test takes about 20 minutes.</u>

<u>Total time for testing visit 1 is approximately 90 minutes</u>

<u>Exercise Training (Visits 3 through 14 for Exercise Training Group)</u>

If you are randomized into the exercise group you will be asked to complete 4 exercise sessions per week for 3 weeks for a total of 12 exercise sessions. These training sessions will include 2 high-intensity interval exercise training session and 2 continuous steady-state exercise training sessions per week. All exercise will be conducted on a treadmill. Training days and times will be flexible, according to your schedule.

- <u>Continuous Exercise Training (2 times per week):</u>
  - 30 minutes at a heart rate corresponding to 75% of your maximum aerobic capacity determined from the baseline treadmill test. . You will have a 5-minute warm-up and 5-minute cool-down at 50-60% of your maximum heart rate.
  -
- <u>High-intensity Interval Exercise Training (2 times per week):</u>
  - Once per week you will perform the following routine:
    - 5-minute warm up at 50-60% of your maximum heart rate
    - Ten 1-minute intervals at 90-95% your maximum heart rate separated by 1 minute of exercise at a low intensity
    - 5 minute cool down at 50-60% of your maximum heart rate
  - Once per week you will perform the following routine:
    - 5-minute warm up at 50-60% of your maximum heart rate
    - Four 4-minute intervals at 90-95% of your maximum heart rate separated by 3 minutes of exercise at a low intensity (about 60% of maximum heart rate)
    - 5 minute cool down at 50-60% of your maximum heart rate

Total time for each exercise training visit is approximately 45 minutes, including warm-up and cool-down.

<u>Doughnut Pickup (Visits 2 through 11 for Control Group; Visits 3, 4, 6, 7, 8, 10, 11, 12 and 13 for Exercise Training Group)</u>

You will be asked to consume 2 doughnuts per day, 6 days per week, for 3 weeks. Doughnuts will be purchased by the study researchers at a local Dunkin Donuts on Monday, Wednesday and Friday morning of each week. You will be required to come to the ISTB3 laboratory on Monday, Wednesday, and Friday of each week for doughnut pickup. This visit can be part of your regular exercise training visit if you are in the Exercise Training group. On each occasion you will be given 4 doughnuts to consume over the next two days. You will be provided a menu of doughnut options so that you will be able to choose the types of doughnuts that you would like most to consume. You may consume the doughnuts at any time of the day that you choose. We ask that you not consume all 4 doughnuts in one day.

Total time commitment for completion of the study is approximately 5 hours and 15 minutes if you are in the Control Group and approximately 13.5 hours if you are in the Exercise Training Group.

RISKS

Research studies often involve some risks. The risks of exercise include local muscle soreness, abnormal changes in blood pressure, nausea, faintness, dizziness, irregular heartbeats (rare), and, in very rare instances, heart attack.

You will be monitored by trained investigators and if there are any adverse effects, the exercise testing or the exercise session will be halted. All exercise testing procedures will comply with the guidelines for exercise test administration as recommended by the American College of Sports Medicine and required by the Healthy Lifestyles Research Center at Arizona State University. You will be asked not to attempt any exercise that you feel is beyond your physical abilities. If you experience discomfort, feel you are unable to continue or wish to stop an exercise at any point, you are requested to inform the investigator immediately.

The blood draw involves a needle puncture in your forearm and hence may lead to some discomfort as well as a slight risk of infection. These will be minimized by using standard procedures for controlling blood-borne pathogens as well as properly cleaning the needle insertion site. Other possible risks of a blood draw include dizziness, fainting, nausea, and vomiting. All blood draws will be conducted while you are seated to ensure your safety in case any of these possible side effects occur.

There is a possible chance of weight gain as a result of potentially higher daily caloric intake. Additionally, a fat-sugar supplemented diet may lead to impairment of arterial function and blood pressure.

As with any research, there is some possibility that you may be subject to risks that have not yet been identified.

BENEFITS

Although there may be no direct benefits to you, you will be provided information on your test results and the study results if you would like us to contact you when the study is complete.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Gaesser will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

WITHDRAWAL PRIVILEGE

It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled. Your participation is voluntary and if you decide not to participate or decide to withdraw from the study it will not affect your grade, treatment, care, employment status.

COSTS AND PAYMENTS

All study procedures will be provided to you at no cost to you. If you are in the Control Group you will be paid $50 (cash) for completion the study, including both testing visits and successful pickup of doughnuts on three days each week. If you are in the Exercise Training Group you will be paid $100 (cash) for completion of the study, including both testing visits and all exercise training sessions.. You will also receive 3 dozen free donuts to consume during the 3 weeks of testing. Partial payment will be made in the following manner if you only complete some of the visits.

- Control Group: Completion of Baseline Testing but failure to complete final visit testing:$25
- Exercise Training Group: Completion of Baseline testing and some/all of the exercise training session, but failure to complete final visit testing: $50

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury. In the event of a medical emergency first aid will be administered and if necessary, 911 will be called.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Glenn Gaesser, 500 N 3rd ST, Phoenix, AZ 85004; 602-827-2283; 480-727-1884.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study

_____ _____ _____

Subject's Signature Printed Name Date

_____ _____

Contact phone number E-mail

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

APPENDIX F

DOUGHNUT MENU

Participant # _____

Please Select **4** Doughnuts from each Category:

Category 1			
	QTY		QTY
Apple n Spice		Jelly Filled	
Bavarian Kreme		Lemon Filled	
Fall Harvest		Maple Frosted	
French Cruller		Strawberry Frosted	
Glazed			

Category 2			
	QTY		QTY
Blueberry Cake		Old Fashioned Cake	
Boston Kreme		Coconut	
Boston Scream		Powdered Cake	
Cinnamon Cake		Vanilla Cocoa Kreme	

Category 3			
	QTY		QTY
Chocolate Frosted Cake		Glazed Cake	
Chocolate Glazed Cake		Glazed Chocolate Cake	
Chocolate Kreme Filled		Pumpkin	
Chocolate Long John		Red Velvet Cake	
Double Chocolate Cake		Vanilla Kreme Filled	
Éclair			

APPENDIX G

STATISTICAL ANALYSIS OUTPUT

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Group		Statistic	df	Sig.	Statistic	df	Sig.
Age	1	.212	6	.200	.933	6	.607
	2	.209	7	.200	.878	7	.218
Height	1	.218	6	.200	.919	6	.496
	2	.328	7	.022	.736	7	.009
WeightPre	1	.194	6	.200	.888	6	.305
	2	.179	7	.200	.943	7	.661
WeightPost	1	.210	6	.200	.873	6	.237
	2	.174	7	.200	.949	7	.721
WeightDelt	1	.180	6	.200	.921	6	.514
	2	.263	7	.152	.908	7	.383
BodyFatPre	1	.230	6	.200	.927	6	.556
	2	.236	7	.200	.884	7	.246
BodyFatPost	1	.258	6	.200	.884	6	.286
	2	.201	7	.200	.924	7	.497
BodyFatDelta	1	.198	6	.200	.898	6	.364
	2	.234	7	.200	.866	7	.171
FatMassPre	1	.244	6	.200	.933	6	.600
	2	.279	7	.105	.811	7	.053
FatMassPost	1	.254	6	.200	.896	6	.348
	2	.246	7	.200	.859	7	.148
FatMassDelta	1	.172	6	.200	.949	6	.730
	2	.140	7	.200	.973	7	.922
SystolicPre	1	.299	6	.102	.841	6	.134
	2	.262	7	.159	.881	7	.232
DiastolicPre	1	.202	6	.200	.956	6	.785
	2	.223	7	.200	.901	7	.338
SystolicPost	1	.195	6	.200	.915	6	.470
	2	.172	7	.200	.934	7	.587
DiastolicPost	1	.235	6	.200	.895	6	.344
	2	.211	7	.200	.875	7	.203

a. Lilliefors Significance Correction

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Group		Statistic	df	Sig.	Statistic	df	Sig.
VO2maxPre	1	.241	6	.200	.936	6	.629
	2	.171	7	.200	.980	7	.961
VO2maxPost	1	.261	6	.200	.852	6	.162
	2	.178	7	.200	.956	7	.786
VO2maxDelta	1	.196	6	.200	.905	6	.403
	2	.217	7	.200	.866	7	.170
RER	1	.198	6	.200	.948	6	.728
	2	.229	7	.200	.941	7	.652
RERPost	1	.290	6	.125	.840	6	.131
	2	.172	7	.200	.967	7	.873
HRPRE	1	.237	6	.200	.868	6	.217
	2	.334	7	.018	.831	7	.081
HRPost	1	.243	6	.200	.885	6	.293
	2	.145	7	.200	.980	7	.959
TTE	1	.192	6	.200	.905	6	.406
	2	.186	7	.200	.953	7	.756
TTEPOST	1	.174	6	.200	.945	6	.701
	2	.263	7	.155	.906	7	.366
Wattage	1	.195	6	.200	.911	6	.441
	2	.160	7	.200	.956	7	.788
WattagePost	1	.179	6	.200	.944	6	.690
	2	.278	7	.108	.898	7	.319
BaseDiamPre	1	.272	6	.187	.889	6	.311
	2	.213	7	.200	.952	7	.750
PeakDiamPre	1	.312	6	.069	.855	6	.172
	2	.198	7	.200	.974	7	.928
FMDPre	1	.211	6	.200	.955	6	.782
	2	.130	7	.200	.981	7	.963
BaseDiamPost	1	.175	6	.200	.923	6	.525
	2	.313	7	.036	.845	7	.111
PeakDiamPost	1	.190	6	.200	.977	6	.934
	2	.234	7	.200	.914	7	.422
FMDPost	1	.234	6	.200	.849	6	.153
	2	.186	7	.200	.968	7	.883
FMDDelta	1	.257	6	.200	.867	6	.216
	2	.340	7	.014	.720	7	.006

a. Lilliefors Significance Correction

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Group		Statistic	df	Sig.	Statistic	df	Sig.
FMDPreAdjBase	1	.272	6	.187	.889	6	.311
	2	.213	7	.200	.952	7	.750
FMDPostAdjBase	1	.272	6	.187	.889	6	.311
	2	.213	7	.200	.952	7	.750
FMDAdjDelta	1	.272	6	.187	.889	6	.311
	2	.213	7	.200	.952	7	.750
Calories	1	.265	6	.200	.869	6	.223
	2	.164	7	.200	.962	7	.838
Totalfat	1	.164	6	.200	.986	6	.976
	2	.227	7	.200	.927	7	.529
Satfat	1	.190	6	.200	.968	6	.879
	2	.339	7	.015	.780	7	.026
Sugar	1	.175	6	.200	.936	6	.629
	2	.241	7	.200	.854	7	.133
CHOLpre	1	.263	6	.200	.941	6	.664
	2	.150	7	.200	.987	7	.987
CHOLpost	1	.270	6	.198	.843	6	.139
	2	.190	7	.200	.948	7	.708
CHOLdelta	1	.218	6	.200	.934	6	.609
	2	.188	7	.200	.915	7	.432
GLU2pre	1	.231	6	.200	.896	6	.353
	2	.150	7	.200	.943	7	.661
GLU2post	1	.203	6	.200	.947	6	.715
	2	.150	7	.200	.962	7	.837
GLU2delta	1	.195	6	.200	.978	6	.943
	2	.198	7	.200	.895	7	.301
HDLpre	1	.272	6	.187	.809	6	.070
	2	.256	7	.181	.851	7	.125
HDLpost	1	.282	6	.147	.877	6	.256
	2	.183	7	.200	.915	7	.435
HDLdelta	1	.256	6	.200	.898	6	.365
	2	.224	7	.200	.904	7	.354
LDLpre	1	.237	6	.200	.863	6	.200
	2	.137	7	.200	.970	7	.897
LDLpost	1	.178	6	.200	.940	6	.656
	2	.200	7	.200	.920	7	.466

a. Lilliefors Significance Correction

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Group		Statistic	df	Sig.	Statistic	df	Sig.
LDLdelta	1	.222	6	.200	.867	6	.216
	2	.162	7	.200	.987	7	.987
TRIGpre	1	.319	6	.056	.702	6	.007
	2	.255	7	.190	.924	7	.504
TRIGpost	1	.221	6	.200	.896	6	.352
	2	.131	7	.200	.971	7	.905
TRIGdelta	1	.276	6	.169	.804	6	.064
	2	.190	7	.200	.955	7	.774
hsCRPpre	1	.259	6	.200	.840	6	.131
	2	.386	7	.002	.590	7	.000
hsCRPpost	1	.247	6	.200	.869	6	.223
	2	.356	7	.008	.667	7	.002
hsCRPdelta	1	.325	6	.047	.731	6	.013
	2	.443	7	.000	.632	7	.001
NOpre	1	.175	6	.200	.915	6	.468
	2	.185	7	.200	.959	7	.811
NOpost	1	.165	6	.200	.984	6	.970
	2	.252	7	.200	.858	7	.144
NOdelta	1	.198	6	.200	.932	6	.599
	2	.144	7	.200	.985	7	.979
AOXpre	1	.214	6	.200	.921	6	.510
	2	.228	7	.200	.885	7	.248
AOXpost	1	.253	6	.200	.888	6	.308
	2	.192	7	.200	.958	7	.804

a. Lilliefors Significance Correction

Change in Body Weight (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
WeightDelta	Exercise	10	-.050000	1.2695098	.4014542
	Control	9	1.691556	2.0192692	.6730897

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
WeightDelta	Equal variances assumed	.872	.363	-2.277	17	.036	-1.7415556	.7649876	-3.3555382	-.1275729
	Equal variances not assumed			-2.222	13.217	.044	-1.7415556	.7837189	-3.4318513	-.0512599

Change in Flow Mediated Dilation (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
FMDDelta	Exercise	9	1.25333	4.274541	1.424847
	Control	9	1.19444	2.913473	.971158

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
FMDDelta	Equal variances assumed	1.109	.308	.034	16	.973	.058889	1.724337	-3.596541	3.714319
	Equal variances not assumed			.034	14.114	.973	.058889	1.724337	-3.636655	3.754433

Change in VO_{2max} (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
VO2maxDelta	Exercise	10	.230500	.2089185	.0660658
	Control	9	-.022222	.1054488	.0351496

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
VO2maxDelta	Equal variances assumed	2.826	.062	3.267	17	.005	.2527222	.0773490	.0895301	.4159143
	Equal variances not assumed			3.377	13.591	.005	.2527222	.0748344	.0917643	.4136802

Change in Body Fat Percentage (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
BodyFatDelta	Exercise	9	-.255556	1.4116578	.4705526
	Control	8	1.500000	1.1058287	.3909695

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
BodyFatDelta	Equal variances assumed	.843	.373	-2.827	15	.013	-1.7555556	.6210339	-3.0792580	-.4318531
	Equal variances not assumed			-2.870	14.798	.012	-1.7555556	.6117817	-3.0610880	-.4500231

Change in Total Cholesterol (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
CHOLdelta	Exiercise	10	6.50000	16.372402	5.177408
	Control	9	-4.11111	11.634479	3.878160

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
CHOLdelta	Equal variances assumed	.452	.511	1.611	17	.126	10.611111	6.588383	-3.289162	24.511384
	Equal variances not assumed			1.640	16.197	.120	10.611111	6.468824	-3.088670	24.310892

Change in Blood Glucose (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
GLU2delta	Exercise	10	-.10000	6.640783	2.100000
	Control	9	1.33333	4.062019	1.354006

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
GLU2delta	Equal variances assumed	1.319	.267	-.559	17	.583	-1.433333	2.562819	-6.840408	3.973741
	Equal variances not assumed			-.574	15.102	.575	-1.433333	2.498666	-6.755979	3.889312

Change in HDL Cholesterol (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
HDLdelta	Exercise	10	4.70000	6.929005	2.191144
	Control	9	-.55556	7.617597	2.539199

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
HDLdelta	Equal variances assumed	.047	.830	1.575	17	.134	5.255556	3.336285	-1.783391	12.294502
	Equal variances not assumed			1.567	16.311	.136	5.255556	3.353900	-1.843396	12.354507

Change in LDL Cholesterol (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
LDLdelta	Exercise	9	2.60556	12.830132	4.276711
	Control	9	-2.51444	11.104841	3.701614

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
LDLdelta	Equal variances assumed	.734	.404	.905	16	.379	5.120000	5.656165	-6.870533	17.110533
	Equal variances not assumed			.905	15.678	.379	5.120000	5.656165	-6.890609	17.130609

Change in Triglycerides (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
TRIGdelta	Exercise	7	-16.02857	29.726504	11.235562
	Control	9	15.07778	24.139614	8.046538

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
TRIGdelta	Equal variances assumed	.011	.917	-2.314	14	.036	-31.106349	13.444272	-59.941444	-2.271254
	Equal variances not assumed			-2.251	11.470	.045	-31.106349	13.819719	-61.371937	-.840762

Change in High Sensitivity C-reactive Protein (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
hsCRPdelta	Exercise	9	-.03444	.627477	.209159
	Control	9	.77111	2.560852	.853617

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
hsCRPdelta	Equal variances assumed	2.467	.136	-.917	16	.373	-.805556	.878869	-2.668674	1.057562
	Equal variances not assumed			-.917	8.957	.383	-.805556	.878869	-2.795145	1.184034

Change in Nitric Oxide Availability (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
NOdelta	Exercise	9	4.33269	8.769026	2.923009
	Control	9	-4.29953	3.398838	1.132946

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
NOdelta	Equal variances assumed	2.858	.110	2.754	16	.014	8.632214	3.134892	1.986541	15.277888
	Equal variances not assumed			2.754	10.351	.020	8.632214	3.134892	1.679181	15.585247

Change in Antioxidant Capacity (Exercise vs. Control)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
AOXdelta	Exercise	9	.0573	.44769	.14923
	Control	9	.1805	.32414	.10805

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
AOXdelta	Equal variances assumed	.024	.880	-.669	16	.513	-.12320	.18424	-.51377	.26736
	Equal variances not assumed			-.669	14.579	.514	-.12320	.18424	-.51689	.27048

APPENDIX H

SUBJECT DATA

Group	Subject	Age	Height	WeightPre	WeightPost	WeightDelt	BMI
1	2	24	183	67.78	67.92	0.14	20.24
1	3	21	176	75.76	74.39	-1.38	24.46
1	6	23	176	76.61	77.64	1.02	24.73
1	7	22	168	64.15	61.18	-2.97	22.73
1	11	25	173.9	73.97	74.79	0.82	24.46
1	16	28	175.5	69.11	69.17	0.05	22.44
1	17	19	172	68.40	68.52	0.12	23.12
1	18	22	171	85.71	86.62	0.92	29.31
1	20	23	177.1	86.90	86.61	-0.29	27.71
1	21	24	187.8	74.52	75.58	1.06	21.13
2	1	29	179	87.80	89.40	1.60	27.40
2	4	24	181	83.41	88.56	5.16	25.46
2	5	25	181	79.39	82.46	3.07	24.23
2	8	20	181.5	71.37	73.28	1.91	21.67
2	9	26	181	123.63	122.36	-1.27	37.74
2	10	19	164.25	55.21	56.67	1.47	20.46
2	12	30	179.2	60.51	63.25	2.74	18.84
2	14	22	170.5	55.60	54.34	-1.26	19.13
2	15	30	174.5	92.72	94.53	1.81	30.45
	AVE G1	23.100	176.030	74.290	74.240	-0.050	24.032
GROUP	**SD G1**	2.424	5.765	7.482	8.090	1.270	2.791
DATA	**AVE G2**	25.000	176.883	78.846	80.538	1.692	25.042
	SD G2	4.153	6.000	21.767	21.502	2.019	6.150
	AVE						
TOTALS	**AGE**	24.000	176.434	76.448	77.223	0.775	24.510
	SD AGE	3.399	5.728	15.622	15.768	1.848	4.580

Group	Subject	BodyFatPre	BodyFatPost	BodyFatDelta	FatMassPre	FatMassPost
1	2	5.1	6	0.90	3.44	4.084
1	3	11.3	10.8	-0.50	8.58	8.025
1	6	24.9	23.9	-1.00	19.05	18.533
1	7	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
1	11	20.5	22.6	2.10	15.16	16.891
1	16	10.9	10.2	-0.70	7.57	7.038
1	17	12.2	10.2	-2.00	8.32	6.971
1	18	24.6	25.4	0.80	21.11	22.009
1	20	31.8	29.6	-2.20	27.81	25.614
1	21	15.3	15.6	0.30	11.40	11.76
2	1	23	24.5	1.50	20.18	21.895
2	4	27.8	30.2	2.40	23.22	26.719
2	5	17.6	19.5	1.90	14.00	15.88
2	8	14.8	16.3	1.50	10.53	11.633
2	9	50.5	50.1	-0.40	62.48	61.331
2	10	9.7	9.8	0.10	5.35	5.476
2	12	12.3	14.7	2.40	7.44	9.301
2	14	#NULL!	#NULL!	#NULL!	#NULL!	#NULL!
2	15	28	30.6	2.60	25.99	28.919
	AVE G1	17.400	17.144	-0.256	13.604	13.436
GROUP	**SD G1**	8.568	8.381	1.412	7.810	7.599
DATA	**AVE G2**	22.963	24.463	1.500	21.146	22.644
	SD G2	13.050	12.702	1.106	18.277	17.700
	AVE					
TOTALS	**AGE**	20.018	20.588	0.571	17.153	17.769
	SD AGE	10.927	10.949	1.532	13.846	13.725

Group	Subject	FatMassDelta	SystolicPre	DiastolicPre	SystolicPost	DiastolicPost
1	2	0.65	113	78	118	78
1	3	-0.55	120	75	115	72
1	6	-0.52	132	63	122	68
1	7	#NULL!	112	66	125	76
1	11	1.73	117	77	117	66
1	16	-0.53	117	70	130	78
1	17	-1.35	119	82	113	70
1	18	0.90	133	80	131	78
1	20	-2.19	132	90	137	90
1	21	0.36	125	86	129	90
2	1	1.72	135	92	143	94
2	4	3.50	127	74	122	85
2	5	1.88	123	81	117	73
2	8	1.11	114	65	115	65
2	9	-1.15	126	68	122	69
2	10	0.13	113	68	113	67
2	12	1.86	129	84	127	77
2	14	#NULL!	118	66	112	63
2	15	2.93	131	85	130	94

	AVE G1	-0.167	122.000	76.700	123.700	76.600
GROUP	**SD G1**	1.203	7.986	8.525	7.959	8.276
DATA	**AVE G2**	1.498	124.000	75.889	122.333	76.333
	SD G2	1.483	7.632	9.867	9.899	12.031

	AVE					
TOTALS	**AGE**	0.616	122.947	76.316	123.053	76.474
	SD AGE	1.556	7.670	8.932	8.702	9.930

Group	Subject	VO2Pre	VO2Post	VO2Delta	RERpre	RERPost	HRPRE	HRPost
1	2	2.99	3.64	0.65	1.2	1.22	192	191
1	3	2.8	3.28	0.48	1.24	1.24	198	196
1	6	2.94	3.01	0.07	1.25	1.24	195	191
1	7	3.59	3.63	0.04	1.3	1.22	190	193
1	11	3.19	3.32	0.13	1.25	1.23	181	173
1	16	3.51	3.62	0.11	1.2	1.22	177	167
1	17	2.98	3.05	0.07	1.16	1.19	174	182
1	18	3.11	3.3	0.19	1.14	1.21	179	188
1	20	2.63	3.05	0.42	1.28	1.24	176	169
1	21	3.14	3.29	0.15	1.23	1.23	188	182
2	1	2.9	2.97	0.07	1.15	1.23	183	181
2	4	2.66	2.67	0.01	1.13	1.21	183	174
2	5	2.34	2.34	0	1.22	1.29	178	178
2	8	2.88	2.69	-0.19	1.33	1.24	184	176
2	9	2.48	2.55	0.07	1.2	1.23	165	166
2	10	2.99	2.81	-0.18	1.21	1.24	188	191
2	12	2.14	2.07	-0.07	1.08	1.25	175	158
2	14	2.14	2.13	-0.01	1.13	1.23	166	183
2	15	3.4	3.5	0.1	1.19	1.22	186	186
	AVE G1	3.088	3.319	0.231	1.225	1.224	185.000	183.200
GROUP	**SD G1**	0.294	0.243	0.209	0.050	0.016	8.628	10.412
DATA	**AVE G2**	2.659	2.637	-0.022	1.182	1.238	178.667	177.000
	SD G2	0.424	0.442	0.105	0.072	0.023	8.426	10.137
	AVE							
TOTALS	**AGE**	2.885	2.996	0.111	1.205	1.231	182.000	180.263
	SD AGE	0.414	0.489	0.209	0.064	0.020	8.907	10.487

Group	Subject	TTEpre	TTEPOST	Wattage	WattagePost	PkDiamPre	PkDiamPost
1	2	8:44:00	9:13:00	301	316	0.36	0.38
1	3	5:54:00	6:52:00	219	247	0.40	0.44
1	6	7:20:00	7:38:00	256	271	0.40	0.40
1	7	9:05:00	9:35:00	316	331	0.40	0.44
1	11	6:50:00	7:45:00	249	271	0.45	0.45
1	16	8:53:00	8:36:00	308	301	0.41	0.45
1	17	6:26:00	7:57:00	234	279	0.40	0.40
1	18	7:21:00	8:15:00	260	294	0.46	0.50
1	20	6:02:00	6:46:00	226	249	0.47	0.45
1	21	8:25:00	9:05:00	294	315	0.40	0.42
2	1	7:26:00	8:53:00	264	309	0.43	0.45
2	4	5:41:00	5:28:00	211	204	0.38	0.41
2	5	6:22:00	6:08:00	234	229	0.43	0.50
2	8	6:30:00	5:11:00	241	196	0.37	0.41
2	9	4:38:00	5:17:00	181	203	0.42	0.39
2	10	7:26:00	7:23:00	263	263	0.32	0.32
2	12	4:03:00	4:05:00	167	167	0.42	0.42
2	14	4:29:00	4:27:00	179	175	0.32	0.35
2	15	8:27:00	8:22:00	303	294	0.47	0.46

GROUP DATA	**AVE G1**	0.312	0.340	266.300	287.400	0.415	0.432
	SD G1	0.050	0.040	35.793	28.722	0.035	0.034
	AVE G2	0.255	0.256	227.000	226.667	0.397	0.411
	SD G2	0.063	0.071	46.046	51.091	0.054	0.055
TOTALS	**AVE AGE**	0.285	0.300	247.684	258.632	0.406	0.422
	SD AGE	0.063	0.070	44.602	50.431	0.045	0.046

Group	Subject	PeakDELTA	BaseDiamPre	BaseDiamPost	BaselineDELTA	FMDPre
1	2	0.02	0.36	0.37	0.01	0.84
1	3	0.04	0.40	0.42	0.02	-0.50
1	6	0.01	0.38	0.39	0.02	5.60
1	7	0.04	0.38	0.43	0.05	5.26
1	11	-0.01	0.43	0.44	0.01	5.36
1	16	0.04	0.41	0.43	0.03	1.23
1	17	0.00	0.37	0.38	0.01	8.15
1	18	0.03	0.44	0.48	0.04	5.94
1	20	-0.02	0.42	0.44	0.01	10.64
1	21	0.01	0.37	0.40	0.03	9.78
2	1	0.01	0.40	0.41	0.01	7.96
2	4	0.03	0.36	0.38	0.03	7.30
2	5	0.06	0.39	0.45	0.07	11.57
2	8	0.04	0.38	0.40	0.02	-1.59
2	9	-0.03	0.39	0.34	-0.05	8.29
2	10	0.00	0.31	0.32	0.02	4.26
2	12	0.00	0.40	0.39	-0.01	6.80
2	14	0.03	0.31	0.32	0.00	0.96
2	15	-0.01	0.45	0.44	0.00	6.28
	AVE G1	0.02	0.39	0.42	0.02	5.23
GROUP	**SD G1**	0.02274887	0.028795254	0.032380378	0.013543756	3.759519
DATA	**AVE G2**	0.01	0.37	0.38	0.01	5.76
	SD G2	0.02950894	0.044269377	0.050059964	0.030384115	4.000835
TOTALS	**AVE AGE**	0.016	0.385	0.401	0.016	5.481
	SD AGE	0.025	0.037	0.044	0.023	3.775

Group	Subject	FMDPost	FMDDelta	Diet Intake	Calories	Totalfat	Satfat	Sugar
1	2	2.73	1.89	2897	11640	702	324	546
1	3	4.49	4.99	2319	11580	720	324	462
1	6	2.80	-2.80	2201	11940	756	345	522
1	7	3.50	-1.77	2604	11640	702	318	540
1	11	2.53	-2.83	2130	11850	672	303	531
1	16	4.61	3.37	2810	11400	720	324	456
1	17	3.94	-4.22	2920	12000	669	303	597
1	18	4.42	-1.52	1910	11550	627	279	579
1	20	2.99	-7.65	2245	11460	672	300	564
1	21	5.82	-3.96	2518	11340	642	291	564
2	1	9.05	1.09	2854	11850	681	309	519
2	4	7.05	-0.25	2705	11880	618	264	552
2	5	9.69	-1.88	2690	11520	696	312	534
2	8		1.59	2505	11580	702	309	441
2	9	14.54	6.25	2412	11760	666	303	585
2	10	-0.63	-4.89	2752	11760	714	327	534
2	12	7.65	0.85	2580	11670	684	309	549
2	14	10.76	9.80	1985	11520	687	306	498
2	15	4.52	-1.75	2008	11730	669	306	546

GROUP DATA	**AVE G1**	3.78	-1.45	2455.400	11640.000	688.200	311.100	536.100
	SD G1	1.06112	3.831513	348.576	224.944	39.120	19.393	46.369
	AVE G2	7.83	1.20	2499.000	11696.667	679.667	305.000	528.667
	SD G2	4.49767	4.431717	313.926	133.697	27.663	16.837	40.632
TOTALS	**AVE AGE**	5.581	-0.193	2476.053	11666.842	684.158	308.211	532.579
	SD AGE	3.634	4.233	324.118	184.633	33.533	17.996	42.701

Group	Subject	CHOLpre	CHOLpost	CHOLdelta	HDLpre	HDLpost	HDLdelta
1	2	129	121	-8	43	43	0
1	3	108	110	2	36	39	3
1	6	202	185	-17	38	43	5
1	7						0
1	11	171	199	28	39	47	8
1	16	148	153	5	49	56	7
1	17	140	178	38	64	85	21
1	18	138	136	-2	68	75	7
1	20	172	186	14	67	63	-4
1	21	171	176	5	53	53	0
2	1	207	194	-13	61	56	-5
2	4	150	130	-20	38	54	16
2	5	198	198	0	58	49	-9
2	8	75	81	6	40	39	-1
2	9	139	143	4	49	46	-3
2	10	116	113	-3	42	43	1
2	12	158	136	-22	70	61	-9
2	14	118	118	0	55	57	2
2	15	176	187	11	37	40	3
							0
	AVE G1	153.222	160.444	7.222	50.778	56.000	5.222222
GROUP	**SD G1**	28.350	31.714	17.196	12.863	15.684	2.821866
DATA	**AVE G2**	145.125	139.125	-6.000	51.625	50.625	-1
	SD G2	43.676	39.808	10.863	11.326	7.615	-3.71094
							0
TOTALS	**AVE AGE**	150.889	152.444	1.556	50.389	52.722	2.333333
	SD AGE	34.918	36.241	15.390	11.917	12.527	0.609643

Group	Subject	LDLpre	LDLpost	LDLdelta	TRIGpre	TRIGpost	TRIGdelta
1	2	82.21	75.89	-6.32	57.43	52.75	-4.68
1	3	64.91	64.09	-0.82	44.73	56.85	12.12
1	6	146.83	132.47	-14.36	201.98	123.24	-78.74
1	7						
1	11	110.97	128.37	17.4	222.84	269.49	46.65
1	16	80.11	75.54	-4.57	137.1	247.14	110.04
1	17	70.91	90.44	19.53	59.5	38.69	-20.81
1	18	59.24	50.11	-9.13	87.98	80.42	-7.56
1	20	103.99	122.16	18.17	62.72	65.48	2.76
1	21	108.3	111.85	3.55	131.96	116.67	-15.29
2	1	135.9	132.91	-2.99	75.28	93.21	17.93
2	4	94.1	70.44	-23.66	114.46	86.79	-27.67
2	5	121.66	127	5.34	143.93	191.28	47.35
2	8	26.25	26.84	0.59	68.68	115.27	46.59
2	9	79.59	84.95	5.36	95.86	106.32	10.46
2	10	64.81	60.05	-4.76	75.89	71.15	-4.74
2	12	83.29	71.73	-11.56	58.16	48.77	-9.39
2	14	55.31	49.35	-5.96	55.95	86.91	30.96
2	15	122.87	137.88	15.01	128.53	120.97	-7.56
	AVE G1	91.941	94.547	2.606	111.804	116.748	4.943
GROUP	**SD G1**	27.984	30.151	12.830	65.836	85.220	51.436
DATA	**AVE G2**	82.614	77.909	-4.705	86.026	99.963	13.936
	SD G2	35.345	36.491	9.570	30.374	42.180	27.057
	AVE						
TOTALS	**AGE**	89.514	89.559	0.046	101.277	109.522	8.246
	SD AGE	31.207	34.503	11.935	51.297	65.027	39.777

Group	Subject	hsCRPpre	hsCRPpost	hsCRPdelta	GLU2pre	GLU2post	GLU2delta
1	2	0.17	0.35	0.18	107	94	-13
1	3	2.7	1.1	-1.6	83	91	8
1	6	0.58	0.33	-0.25	87	84	-3
1	7						0
1	11	0.11	0.52	0.41	91	91	0
1	16	0.15	0.61	0.46	82	91	9
1	17	1.34	1.33	-0.01	80	82	2
1	18	0.16	0.43	0.27	76	73	-3
1	20	0.72	0.72	0	90	84	-6
1	21	0.32	0.55	0.23	88	93	5
2	1	0.48	0.7	0.22	89	92	3
2	4	0.41	0.29	-0.12	96	94	-2
2	5	1.07	0.96	-0.11	93	89	-4
2	8	0.31	0.51	0.2	91	99	8
2	9	4.26	2.81	-1.45	99	106	7
2	10	0.13	0.45	0.32	88	89	1
2	12	0.37	7.81	7.44	80	78	-2
2	14	0.15	0.52	0.37	94	94	0
2	15	0.87	0.94	0.07	100	99	-1
	AVE G1	0.694	0.660	-0.034	87.111	87.000	-0.111
GROUP	**SD G1**	0.851	0.342	0.627	8.923	6.819	7.044
DATA	**AVE G2**	0.898	1.756	0.859	91.250	92.625	1.375
	SD G2	1.390	2.576	2.723	5.800	8.141	4.340
TOTALS	**AVE**	0.794	1.163	0.368	89.667	90.167	0.474
	STDEV	1.071	1.758	1.856	7.889	7.868	5.481

Group	Subject	Insulinpre	Insulinpost	InsulinDelta	HOMApre	HOMApost	HOMAdelta
1	2	8.401	7.166	-1.235	2.220	1.663	-0.556
1	3	7.323	7.303	-0.02	1.501	1.641	0.140
1	6	15.142	12.585	-2.557	3.253	2.610	-0.643
1	7			0	0.000	0.000	0.000
1	11	14.295	10.285	-4.01	3.212	2.311	-0.901
1	16	13.585	11.923	-1.662	2.751	2.679	-0.072
1	17	7.185	7.093	-0.092	1.419	1.436	0.017
1	18	16.629	16.011	-0.618	3.121	2.886	-0.235
1	20	8.822	7.95	-0.872	1.960	1.649	-0.312
1	21	8.893	5.156	-3.737	1.932	1.184	-0.748
2	1	7.152	11.367	4.215	1.572	2.582	1.010
2	4	7.95	8.638	0.688	1.884	2.005	0.120
2	5	5.469	5.434	-0.035	1.256	1.194	-0.062
2	8	12.921	23.002	10.081	2.903	5.623	2.719
2	9	10.274	18.844	8.57	2.511	4.932	2.421
2	10	12.55	11.944	-0.606	2.727	2.625	-0.102
2	12	6.682	6.42	-0.262	1.320	1.236	-0.083
2	14	10.52	6.95	-3.57	2.442	1.613	-0.829
2	15	13.2	11.95	-1.25	3.259	2.921	-0.338
	AVE G1	11.142	9.497	-1.645	2.374	2.007	-0.368
GROUP	**SD G1**	3.712	3.459	1.486	0.728	0.619	0.363
DATA	**AVE G2**	9.190	11.575	2.385	2.077	2.726	0.649
	SD G2	2.777	6.301	4.796	0.651	1.674	1.289
TOTALS	**AVE**	10.389	10.557	0.159	2.171	2.252	0.081
	STDEV	3.332	4.814	3.724	0.867	1.303	0.979

Group	Subject	Nopre	Nopost	Nodelta	Aoxpre	Aoxpost	Aoxdelta
1	2	1.61	2.17	0.56	2.18	2.13	-0.05
1	3	1.05	0.56	-0.48	2.05	2.16	0.11
1	6	3.14	1.64	-1.49	2.32	2.68	0.36
1	7						
1	11	26.16	51.87	25.71	2.60	2.66	0.06
1	16	16.76	27.21	10.45	3.22	2.66	-0.56
1	17	3.47	2.88	-0.60	2.77	2.46	-0.31
1	18	2.20	3.06	0.86	2.71	2.68	-0.03
1	20	0.90	3.92	3.02	2.36	3.40	1.03
1	21	8.55	9.52	0.97	3.19	3.10	-0.09
2	1	8.18	1.38	-6.79	1.60	1.97	0.37
2	4	4.22	2.58	-1.64	2.41	2.18	-0.23
2	5	10.49	2.54	-7.95	2.38	2.26	-0.12
2	8	11.23	1.53	-9.70	1.71	2.27	0.56
2	9	6.01	1.57	-4.44	1.91	2.24	0.33
2	10	0.97	2.35	1.38	2.35	2.65	0.30
2	12	4.22	1.79	-2.43	2.36	2.43	0.06
2	14	4.85	1.01	-3.84	2.19	1.96	-0.23
2	15	4.93	1.64	-3.28	2.15	2.74	0.59
	AVE G1	7.092	11.425	4.333	2.601	2.658	0.057
GROUP	**SD G1**	8.780	17.274	8.769	0.417	0.404	0.448
DATA	**AVE G2**	6.272	1.845	-4.426	2.115	2.244	0.129
	SD G2	3.477	0.581	3.618	0.328	0.225	0.304
TOTALS	**AVE**	6.607	6.624	0.017	2.360	2.479	0.119
	STDEV	6.450	12.844	7.834	0.434	0.380	0.384